in preparing the sodium salt of the barbituric acid. Smiley (4) suggested that only 95% of the calculated quantity of sodium hydroxide be used in preparing the salt of the acid. The slight excess of acid then could be filtered off and a product would be obtained which would withstand heating at 100° C. in sealed containers for short periods without decomposition. In this regard it may be pointed out that amines have been used as basic substances to replace the sodium hydroxide. The intention is to replace the strong inorganic alkali by a weaker base. The use of amines is reveiwed in another part of this discussion.

A fall in the pH of solutions of sodium amytal upon aging was reported by Cazzani (16). Fresh solutions have pH values of 9.5 to 9.8. The solutions decompose readily on standing. When the pH falls to 9.3, they become opalescent and cloudy.

b. Temperature

Nielsen (13) found that the rate of decomposition of solutions of sodium phenylethyl barbituric acid varied greatly with temperature. The rate was followed by determining the amount of carbon dioxide and phenylethylacetylurea formed. The results which he obtained are indicated in Table 8.

TABLE 9

EFFECT OF TEMPERATURE ON 10% AQUEOUS SOLUTIONS OF SODIUM PHENYLETHYL BARBITURATE ADJUSTED TO A pH OF 9.4 WITH PHENYLETHYLBARBITURIC ACID

Temperature	Time	Per cent Decomposed
1° C.	2 months	less than 1%
20°	3 weeks	1
39°	1 month	22

A 1:300 solution of sodium phenobarbital, which is kept at 20° C. is 1% decomposed in 5 weeks, according to Nielsen (13).

Tomski and Waller (24) measured the rate of decomposition of solutions of sodium phenobarbital by the Hegland method (25). This method consists of titrating a solution of the barbiturate, to which sodium carbonate has been added, with N/10 silver nitrate until a faint opalescence is formed which does not disappear on shaking. Investigating the effect of heating solutions of different strengths under the same conditions, Tomski and Waller (24) have found that the loss of sodium phenobarbitone increases with increase in concentration. The difference was small, however, and for all practical purposes the loss on heating a 20%, 10% or even a 5% solution is the same, according to these workers. Solutions of 2% to 10% were stored and analyzed periodically. A solution kept for four months (December-March) lost 6% whereas a solution of equal strength when kept for the same length of time (March-June) lost 9% of the phenobarbital sodium. Additional results which were

secured by Tomski and Waller (24) are given in Table 10.

TABLE 10

20% SOLUTIONS OF PHENOBARBITAL SODIUM IN AMPULS HEATED AT 80° C. FOR VARYING LENGTHS OF TIME

Time in minutes	Percentage Loss
30	1.0 to 1.5
60	2.0 to 3.0
120	4.0 to 5.0
180	6.0 to 7.0

A 20% solution therefore loses 6.0 to 7.0% under conditions similar to those prevailing in the process of tyndallization. A solution of the same strength loses 1.0 to 1.5% when sterilized by the emergency method of the British Pharmacopoeia, 1932 (26). Further results obtained by Tomski and Waller (24) are indicated in Tables 11, 12, and 13.

TABLE 11

20% (APPROX.) SOLUTIONS OF PHENOBARBITAL SODIUM IN AMPULS HEATED IN BOILING WATER FOR FIFTEEN MINUTES

Initial Strength	Final Strength	Loss
19.6%	19.3%	1.0%
18.7	18.52	1.0
20.1	19.9	1.0

TABLE 12

20% SOLUTIONS OF PHENOBARBITAL SODIUM EXPOSED TO STEAM AT 100° C. FOR VARIOUS LENGTHS OF TIME

Time in Minutes	Percentage Loss
60	9.5 to 10.5
120	14.5 to 15.5
180	19.5 to 20.5

TABLE 13

20% (APPROX.) SOLUTIONS OF PHENOBARBITAL SODIUM IN AMPULS EXPOSED TO STEAM AT 116° C. FOR THIRTY MINUTES

Initial Strength	Final Strength	Loss
19.6%	16.0%	18.2%
20.1	16.0	20.4
18.7	15.1	19.3

Tomski and Waller (24) obtained a white precipitate of phenylethylacetylurea when a 20% solution of phenobarbital sodium was exposed to any of the methods of sterilization cited above.

Van Leent (27) stated that sterilization of sodium phenobarbital in the dry state at 120° C. yielded a product which gradually became incompletely soluble after this treatment. He suggested that this was due to the action of traces of moisture.

Bailey (4) likewise found that with increasing temperature, greater amounts of precipitate were formed in solutions of sodium diethyl barbiturate. He obtained needle-shaped crystals on autoclaving 7%, 10%, and 14% solutions for thirty minutes at 105° C. Increasingly higher yields were obtained upon autoclaving these solutions at 110°, 115°, and 120° C., the size of the needles varying with the temperature. Upon removing the crystals and subsequent autoclaving an additional quantity of crystals were obtained. At lower temperatures, the yield was influenced by the prolongation of heating. Stich (28) claimed that 20% solutions withstood sterilization in steam at 100° C. He acknowledged that 1 to 5% solutions are hydrolyzed by warming to 100° C. The British Pharmacopoeia, 1932 (28) contains the information that solutions of sodium barbital can be sterilized in an autoclave at 115° C. for 30 minutes or by tyndallization at 80° C. Berasain and Vitali (23) stated that solutions which had been subjected to tyndallization were not suitable for injection. As has been mentioned before, Bailey (4) stated that solutions of the sodium salt which had been prepared by using only 96% of the calculated quantity of sodium hydroxide, would withstand heating at 100° C. for short periods without decomposition.

Bailey (4) felt that the decomposition reaction was probably reversible. The course of the reaction was influenced by the initial concentration of the solution, temperature, and removal of the precipitated product.

Bailey (4) found that a higher yield was produced when the autoclaving was carried out in an open flask than when conducted in a sealed

vessel. This was explained by the escape of carbon dioxide, presumably driving the reaction towards completion by the removal of one of the products from the field of chemical reaction.

From a study of a number of alkyl and aryl substituted malonylurea compounds, Bailey (4) found that their solutions all decomposed at temperatures exceeding 100° C. and that some decomposed at temperatures below the boiling point of the solution. Ruhkopf (29) studied the behavior of substituted barbituric acid during heating at elevated pressures in the absence of alkalies. When pressures up to five atmospheres were used, the substituted barbituric acid was almost completely hydrolyzed with the formation of approximately equal amounts of the substituted acetyl urea and the substituted acetamide. The formation of the amide was favored with increasing pressures, becoming the only product at a pressure of about ten atmospheres in addition to the carbon dioxide and ammonia formed. At a pressure of five atmospheres, salts of strong acids were found to enhance the formation of the substituted acetylurea, whereas salts of weak acids were found to increase the production of the substituted acetamide. Above the latter mentioned pressure, the amount of the acetylurea derivative decreases under any circumstances. Hydrolysis of diethyl barbituric acid in aqueous solution at five atmospheres yielded 47% diethylacetylurea and 40% diethylacetamide. At ten atmospheres, 95% diallylacetamide was formed in the hydrolysis of diallylbarbituric acid in aqueous solution.

3. Stabilization of Barbiturate Solutions

a. Adjustment of Hydrogen Ion Concentration

Serrasin and Vitali (23) found that decomposition of solutions of sodium phenylethyl barbiturate was decreased when their pH value was lower. The same was found true for solutions of sodium diethylbarbiturate by Madsen (3), Bailey (4) and by Tomski and Waller (24).

Schlemmer and Törber (30) claimed that solutions of sodium diethyl barbiturate possessing a pH of 8.92 underwent no hydrolysis within the limit of error of 0.4%, when subjected to the usual conditions of sterilization. Serrasin and Vitali (23) attempted the use of buffers to control the pH. The lowest pH value of their solutions was pH 7. A decrease in the amount of precipitate was noted in solutions of pH less than 9.00, when heated fifteen minutes by steam.

Mesnard (18) suggested the use of sodium bicarbonate as a stabilizer in solutions of sodium phenobartital; its effect was supposedly as follows:

$$C_{12}H_{11}O_3N\ (N\ Na) + H_2O \longrightarrow C_{12}H_{11}O_3N\ (NH) + NaOH$$

$$NaHCO_3 + NaOH \longrightarrow Na_2CO_3 + H_2O$$

$$C_{12}H_{11}O_3N\ (NH) + Na_2CO_3 \longrightarrow C_{12}H_{11}O_3N\ (N\ Na) + NaHCO_3$$

Serrasin and Vitali (23) stated that the use of sodium bicarbonate was unsatisfactory.

b. Amines as Stabilizing Agents

One of the earliest applications of amines to stabilize barbiturate

solutions made its appearance as a commercial product called Somnifene. Somnifene is manufactured by Hoffman-LaRoche and Company (31) and contains in 1 cc. the diethylamine salts of 0.1 Gm. of diethyl barbituric acid and 0.1 Gm. of isopropyl propenyl barbituric acid. In 1921, Le Medicin (32) described it as being soluble and with effects superior to those of the other barbiturate derivatives. Now Somnifene is the isopropylallyl barbiturate of diethylamine. According to Cazzani (17), it can be sterilized by tyndallization at 80° C., has good keeping qualities, and is well tolerated.

In a British Patent (33) it is claimed that a 5 to 10% solution of butylethyl barbituric acid, which is stable at 100° C. and sterilizable by tyndallization, can be made by neutralizing the acid with aminoethanol, diethylaminethanol, and diethylamine. Cazzani (16) stated that keeping qualities of these solutions are good. Piperazine has also been used in an analogous manner and Cazzani (16) indicated that it too yields solutions with good keeping qualities. All of the above solutions are claimed to be well tolerated.

Blok (34) reported that a stable solution of phenobarbital suitable for intramuscular injection can be made by the use of diethylamine. Blok (34) suggested the following formula:

Phenobarbital	10	Gm.
Diethylamine	2.75	Gm.
Petit Mixture q.s. ad	100	cc.

Petit Mixture

Alcohol (90%)	26	Gm.
Glycerin	31	Gm.
Water q.s. ad	100	cc.

The phenobarbital was placed in a sterile flask containing some sterile Petit Mixture. The diethylamine was added and the mixture was shaken and heated at 40° to 60° C. in a closed flask until solution was effected. Sufficient Petit Mixture was added to make 100 cc. Then the solution was filtered, placed in ampule and sterilized for a half hour at 60° C. during two successive days. No appreciable decomposition was found after three months. When heated in flowing steam for one hour, 2% was hydrolyzed. Schulte (35) modified Blok's procedure (34) by neutralizing the barbituric acid with diethylamine dissolved in alcohol and then adding glycerin. In this way, the solution is obtained without the use of heat. Berasain and Vitali (23) subjected Blok's solution (31) to steam heat for fifteen minutes. The solution became colored and an abundant quantity of precipitate was produced. Glycerin and alcohol would be objectionable for injections. The latter authors pointed out that glycerin produces deleterious effects at the site of injection. In addition alcohol produces a disagreeable burning sensation when injected.

Van Leent (27) stated that a solution of sodium barbital is best prepared by dissolving barbital in diethylamine and sterilizing for thirty minutes at 80° to 86° C. on two successive days.

E. R. Squibb and Sons have patented (36) a preparation which is claimed to be stable and which may be sterilized by heating. It is an aqueous solution containing ethyl isopropyl barbituric acid and more than a molecular proportion of diethanolamine.

Meanard (18) stated that sodium phenobarbital in solution hydrolyzed

into phenobarbital and sodium hydroxide, followed by cleavage of the phenobarbital itself due to the action of the sodium hydroxide formed. To prevent this action, Hassard (18) suggested the use of ethanolamine acetate in solutions of this barbiturate. The intention was to have the sodium hydroxide liberate ethanolamine which would form a stable salt with barbituric acid. Berasain and Vitali (33) declared this procedure was unsatisfactory. They found that 10% solutions of this barbiturate prepared with mono-, di, and tri-ethanolamine (or piperazine) were not stable when subjected to steam sterilisation for fifteen minutes.

Nightingale and Morris (37) stated that the amine salts of the 1-phenyl derivative of 5-bromobarbituric acid are less stable than those without the phenyl group. In alcohol 5,5' dibromobarbituric acid reacted rapidly in ethyl alcohol with butylamine to give the relatively stable butylamine salt of 5-bromobartituric acid. Analogous compounds with a 1-phenyl group were unstable. Cold sodium carbonate caused the latter to break up into butylamine and 1-phenyl 5-bromobarbituric acid.

Brolumal is a product of the Italian Drugs Importing Company, Inc. (38) which contains hexamethyltetramine. Each ampul of 2 cc. contains:

Phenylethyl barbituric acid	0.015 Gm.
Organic Bromine	0.010 Gm.
Hexamethyltetramine	0.010 Gm.
Hydroglyceric solution, sterile	q.s.

Brolumal is made for intramuscular and intravenous injection. Eli Lilly and Company (39) claimed that stable solutions of amytal could be made by dissolving amytal in hydroalcoholic mixtures containing a sufficient

quantity of methenamine. Elixir Amytal, Lilly is stated to be such a stable solution. Each fluid ounce contains amytal, 2 gr., in a vehicle containing methenamine, 2 gr. per fluid ounce, glycerin, alcohol, water, and aromatics. The alcohol content is 30%.

Elixir Amytal, Lilly (1%, approx.)

Amytal	gr. iv
Methenamine	gr. iv
Glycerin	
Alcohol	
Water	
Aromatics	q.s. ad 3 i

The alcohol content is 34%.

In a German patent (40) mention is made of the preparation of a soluble compound of phenobarbital by the addition of at least one molecular proportion of diethylenediamine.

Vande Velde (41) studied the influence of neutral salts on the hydrolysis of urea at elevated temperatures. He found that hydrolysis was accelerated by sodium, potassium and phosphate ions and retarded by ammonium ions.

c. Amides as Stabilizing Agents

The use of amides to prepare solutions of barbiturates suitable for injection has appeared in a number of patents (42,43). Unsubstituted amides of the lower aliphatic acids and amides of the lower aliphatic acids with one or two alkyl groups attached to the nitrogen atom are used to convert barbituric acid derivatives such as phenylethyl barbituric acid or cyclohexenyl barbituric acid into water soluble compounds

whose solutions are claimed to be suitable for subcutaneous injection. Specific amides mentioned are monomethyl and monoethyl acetamide.

In a French Patent (44) a description is given of the use of unsubstituted amides of fatty acids, having at least three carbon atoms in the molecule, as auxiliary agents to obtain 20% solutions of barbituric acid derivatives, the 5, 5' substituents of which consist of an aliphatic and a cyclic group. A solvent for cyclohexylethyl barbituric acid is given as:

Propionamide	62
Betaine	3
Water	16%

A solvent for phenylethylbarbituric acid is given as:

Propionamide	60
Acetamide	40
Water	10%

Similar solvents are given in an Austrian Patent (45). A 10% solution of phenylethyl barbiturate in the following solvent is cited:

Acetamide	65
Betaine	5
Water	30%

Addition of a little urea and/or ethyl alcohol may be advantageous, according to the patent.

d. Use of Urethane in Conjunction with Other Agents

A British Patent (46) described the use of a mixture of tertiary chlorobutyl alcohol and urethane as a solvent for substituted barbituric acids. This alcohol and urethane may be mixed in equal parts by weight to give a liquid product which is suitable as a solvent of barbiturates for injection.

The Danish Pharmacopoeia (47) contains a formula entitled "Compound Solution of Phenylethyl barbituric Acid" in which ethylurethane is employed. The formula is as follows:

Phenylethyl barbituric acid	20 Gm.
Amylene Hydrate	28 Gm.
Ethyl Urethane	35 Gm.
Distilled Water	7 Gm.

The solution is prepared by keeping the mixture in a well-closed flask at 20° to 30° C. until dissolved. It is then filtered through a bacteria-proof filter and sterilized. Zwikker (48) claimed that this non-alkaline solution is suitable for injection. Van Leent (27) stated that such compounds as amidopyrine and caffeine may be dissolved in the solution. Whether or not they act as stabilizing agents was not stated. Berassin and Vitali (23) claimed that the Danish formula is unpractical for injection because of the proportion and nature of the ingredients.

Nielsen (49) followed the decomposition of the barbituric acid derivatives contained in Solution Hypnopheni (Ph. Dan., 1933) by measuring the carbon dioxide produced. Storage for a period of one year showed slight decomposition of diethyl barbituric acid, allylisopropyl barbituric acid, and urethane. Nielsen (50) developed the following formula for injection:

Phenylethyl barbituric acid	3.0
Sodium phenylethyl barbiturate	6.72
Urethane	25.0
Alcoholic Spirit (Ph. Dan.,1933)	15.0
Glycerin	12.5
Aq. dest. steril. q.s. ad	100.0 cc.

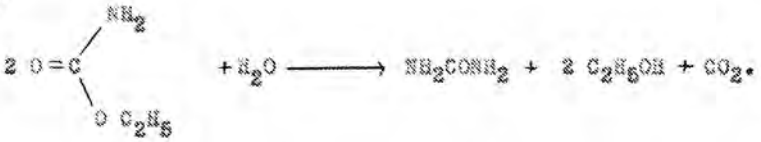
The specific gravity of this preparation at 15° C. was 1.056. In three months at 12° C., about 0.55% of the barbiturates were hydrolyzed, and at 24° C. in the same time 1.35% were hydrolyzed. This decomposition

was one-fifth of that occurring in a 10% aqueous solution of sodium phenobarbital, kept under the same conditions. The solubility of the main decomposition product, phenylethylacetylurea, in the solution was 1% at approximately 22° C. This corresponded to a 12.5% decomposition of the sodium phenobarbital. Therefore, Nielsen (50) reasoned that the preparation could be stored for a long time without any precipitation. After 2 hours warming at 80° C. only the primary decomposition was detected and it amounted to about 0.76% of sodium phenobarbital. The decomposition of urethane ensuring during heating at 80° C. was also determined. The decomposition is represented by the following equations:

(A)



(B)



Nielsen (50) also gave a formula for a 10% solution of phenobarbital, suitable for injection, containing diethylamine. Nielsen's formula (50) is as follows:

Phenobarbital	10.0
Urethane	25.0
Alcoholic Spirit (Ph. Dan., 1933)	15.0
Glycerin	12.5
Diethylamine	20.0
Aq. dest. steril. ad	100 cc.

The specific gravity of this solution at 15° C. was 1.035. Nielsen (50) claimed that this solution was very stable. No hydrolysis was detected after 6 1/2 months storage at 24° C., nor after two hours heating at 80° C. Serasain and Vitali (23) heated this solution for fifteen minutes in steam. It became deeply colored and yielded a precipitate in a few days. They pointed out that Nakieten et al. (61) had found that urethane, in association with some barbituric acid derivatives in narcotic doses, had produced somber effects and alterations in the acid-base equilibrium, tending towards acidosis. The disadvantages of glycerin and alcohol in solutions intended for injection have been mentioned elsewhere.

In a British Patent (52) the claim is made that a stable solution of 5,5 disubstituted barbituric acids can be prepared by dissolving the barbiturate with the aid of at least one molecular proportion of an alkanolamine in the presence of a relatively large quantity of a carbamic ester. For example, 20 Gm. of 5,5 isobutylallyl barbituric acid, 5.5 Gm. of monoethanolamine, and 50 Gm. of urethane are dissolved in 34 cc. of water.

s. Other Nitrogen Compounds as Stabilizing Agents

A British Patent (53) stated that stable solutions of barbiturates for therapeutic use are obtained by adding pyridones or piperidones to their aqueous solutions. The pH of the solutions is 6.0 to 6.6. A solution of allyl isopropyl barbituric acid containing 1-methyl 2-piperidone is specifically mentioned.

Hazleton, Koppanyi, and Lineger (64) employed antipyrine, glycerin,

and alcohol as stabilizing agents for solutions of sodium secondary amyl bromallyl barbiturate. They suggested the following formula:

Sodium secondary amyl bromallyl barbiturate		10 Gm.
Glycerin		10 Gm.
Antipyrine		10 Gm.
Alcohol		10 cc.
Water	q.s. ad	100 cc.

The solution is intended for rectal administration.

Gruber (55, 56, 57) patented the use of urea, urethane, acetamide, and such pyrazolon derivatives as dimethylphenyl pyrazolone for the stabilization of aqueous solutions of barbiturate salts.

f. Glycols as Stabilizing Agents

Page and Coryllos (58) claimed that stable solutions of practically all of the barbiturates could be made by dissolving them in ethylene glycol. Merely the concentration of the solvent had to be varied according to the barbiturate to prepare a stable solution. A solution of sodium amytal is prepared in the following manner: the amount of amytal needed to make a 10% solution is dissolved with very vigorous shaking in the least quantity of 15% sodium hydroxide, without the use of heat. Ethylene glycol is added in sufficient quantity so that its concentration in the final solution will be 15%. The excess sodium hydroxide is neutralized with dilute hydrochloric acid, the solution being shaken vigorously upon the addition of each drop of acid. The shaking is necessary to prevent the separation of isoamyl ethyl barbituric acid in clumps which are difficult to redissolve. When a fine cloudiness remains, the addition of acid is discontinued. A minimum quantity of concentrated ammonium hydroxide is then added quickly to clear the solution. If a

large excess of ammonia is present, it may be quickly boiled off over a free flame. The entire procedure must be performed rapidly to prevent hydrolysis. Berasain and Vitali (23) stated that they found this solution to be stable. They remarked that Oettingen and Jerousch (58) had found ethylene glycol to be toxic, hemolytic, and intensely irritating at the site of injection. Page and Corylios (56) pointed out that isoamylethyl barbituric acid is soluble in ethylene glycol and that it is not essential that the sodium salt be prepared. The ethylene glycol solutions may be heated to boiling to augment the already powerful sterilizing action of ethylene glycol.

In a British Patent (60) and a U. S. Patent (61) it is stated that stable and sterilizable preparations of barbiturate salts can be prepared by the use of alkylene glycols as solvents. The alkali metal salt of the barbituric acid compound is dissolved in the glycol, such as ethylene or propylene glycol, in the absence of any substantial quantity of water. The solution may also be made by dissolving the calculated quantity of an alkali metal in the glycol under anhydrous conditions and then dissolving the free barbituric acid in this solution. Diethyl-, dipropyl-, diallyl-, phenylethyl- and cyclohexenylethyl-barbituric acids and their substitution products were specified in the patent. Dumas (62) stated that he had been informed by certain manufacturers that they were using ethylene and propylene glycols to prepare anhydrous solutions of barbiturates of sufficient stability to permit sterilization.

Berasain and Vitali (23) claimed that the following formula provided solutions stable to sterilization:

Sodium phenobarbital		10 Gm.
Propylene glycol		40 cc.
Distilled water	q.s. ad	100 cc.

The barbiturate was dissolved in the propylene glycol and sufficient water was added to make 100 cc. The solution was sterilized by heating in steam for fifteen minutes. The solutions thus treated were found to be free of aerobic and anaerobic bacteria by inoculating bouillon and agar and observing after 24, 48 and 72 hours. They claimed that the solutions were stable for the length of time observed, namely, six months. The concentration of the propylene glycol is the minimum required to maintain stability. This glycol was similarly used to make solutions of sodium barbital and sodium amytal. They withstood sterilization with steam for 15 minutes. Braun and Gartland (63) demonstrated that propylene glycol is less toxic than glycerin.

It has been reported that propylene glycol may be substituted for glycerin in any non-official article where glycerin itself may be safely used without increasing the toxicity of the resulting product (64).

In a U. S. Patent (65) the use of aliphatic polyhydric alcohols, such as glycerol, or a glycol, is claimed to stabilize aqueous solutions of barbiturate salts.

A German Patent (66) contained a claim that stable solutions of barbiturate salts can be prepared by using an anhydrous glycol, such as ethylene glycol, as a solvent.

g. Miscellaneous Stabilizing Agents

The use of bases weaker than sodium hydroxide to prepare water-soluble compounds of various barbituric acids led Berasain and Vitali (23)

to try calcium saccharosate. To prepare it, 200 cc. of water was added to 10 Gm. of lime. After twenty-four hours had elapsed, the solution was decanted, and a solution of 60 Gm. of sucrose in 200 cc. of distilled water was added to the residue remaining. After another twenty-four hours had elapsed, the calcium saccharosate was obtained by filtration. It was found to be a good solvent for phenobarbital. However, an abundant precipitate, which did not disappear on cooling, was produced when solutions were sterilized with steam for 15 minutes.

Page and Corylles (50) claimed to have prepared a fairly stable solution of sodium amytal, suitable for intravenous injection, by the use of mucilage of acacia. A 20% solution of acacia, in sufficient quantity to make a final concentration of 10%, was added to a solution of amytal just before the excess sodium hydroxide used in dissolving the acid was neutralized by hydrochloric acid. The solution was further stabilized by the addition of sufficient ethylene glycol to make 10%.

An anonymous writer in the Pharmaceutical Journal (67) made the claim that suspensions of phenylethyl barbituric acid in mucilage of tragacanth retained their therapeutic activity for a long time.

Maldi (68) stated that a stable solution of sodium phenobarbital could be prepared by dissolving it in pure glycerin or a mixture of equal parts of glycerin and water. He reported that a 4% solution prepared in this way remained perfectly clear and resisted deterioration on heating to 100° C. Maldi (68) gave the following formula:

Sodium Phenobarbital	20 Gm.
Glycerin	20 Gm.
Alcohol	10 Gm.
Distilled water q.s. ad	100 cc.

Five per cent magnesium sulfate and 10% mannitol are occasionally used to prevent the dissociation of organic salts. Berasain and Vitali (23) found that their use in solutions of sodium phenobarbital failed to give good stabilization.

Bush, Dickinson, and Lamson (69) studied the stability of seconal in paraldehyde. They found that a 30% solution of seconal in paraldehyde retained its original anesthetic activity nearly completely after heating at 120° C. for four hours. Upon intramuscular injection of this solution in some thirty patients, it was found that no discomfort, nor any sign of irritation or injury to tissue was produced. These workers determined the solubility of a number of barbiturates in various substances such as propylene glycol, triacetin, cellosolve, etc. Stability of these solutions was not reported.

B. METHODS OF ASSAYING BARBITURIC ACID DERIVATIVES

1. Procedures Employing Immiscible Solvents

a. The U. S. P. XI and U. S. P. XII Methods (70, 71)

The salt of the barbiturate (barbital sodium, pentobarbital sodium, and phenobarbital sodium) is dissolved in water and then precipitated as the free acid by the addition of diluted hydrochloric acid. The organic acid is extracted with ether. The ethereal solution is evaporated to dryness. The residue is dried to constant weight. In the U. S. P. XI

assay (70), the sample is dried before extraction, whereas in the U. S. P. XII assay (71), the percentage of barbiturate recovered is calculated on a moisture-free basis, employing a sample of known moisture content.

Phenobarbital Tablets and Phenobarbital Sodium Tablets of the U. S. P. XII (71) are freed of such diluents as stearic acid by shaking with a saturated solution of sodium chloride and barium hydroxide. The volatile solvent employed in the extraction of the barbiturate is composed of eight volumes of chloroform and two volumes of ether.

b. Method of Rotondaro

Aspelund and Skoglund (7) separated the decomposition products from deteriorated solutions of various barbiturates by filtration and extraction with ether at various pH levels. This work showed that all aqueous solutions may undergo appreciable decomposition. Analyses employing immiscible solvents require that the barbituric acid be free from its products of decomposition. Rotondaro (72) pointed out that the N. F. VI Method of assaying Elixir of Phenobarbital (73) eliminated the neutral decomposition product, phenylethylacetylurea, by discarding it along with the aromatics. The acidic decomposition products, phenylethylmalonuric acid and phenylethylacetic acid, are not separated from the barbituric acid. The presence of these acids arising from the decomposition of phenobarbital is indicated by the fact that the melting point of the barbituric acid residue is usually low. Rotondaro (72) asserted that the contaminant was phenylethylacetic acid and not glycerin as had been expected by others when assaying Elixir of

of Phenobarbital N. F. VI (73).

Rotondaro (72) carried out an experiment in which he was able to isolate several of the decomposition products of a barbiturate. A solution of phenobarbital in 0.2 N potassium hydroxide was refluxed for five hours. Upon dilution with water, a flocculent precipitate was formed. A portion of the mixture was extracted with chloroform. The residue obtained on evaporating the chloroform extracts was syrupy. However, on dissolving in alcohol and diluting with water, a white, flocculent precipitate separated out. Its melting point was the same as that of phenylethylacetylurea. Another portion of the alkaline solution which had been refluxed was made acid to litmus with dilute hydrochloric acid. Upon adding excess sodium bicarbonate and extracting with chloroform, Rotondaro (72) was able to secure a residue which had the melting point of phenobarbital. The remaining bicarbonate solution was acidified and extracted with chloroform. A syrupy residue was obtained which Rotondaro (72) was unable to crystallize. However, by titrating a solution of the residue in alcohol with 0.1 N sodium hydroxide, a titration equivalent of 206 was obtained. The titration equivalent of a 1:1 mixture of phenylethylacetic acid and phenylethylmalonuric acid is 207.

Rotondaro (72) devised the following method to assay Elixir of Phenobarbital N. F. VI (73):

"A 50 cc. sample was diluted with about 50 cc. of water in a separator, saturated with sodium chloride, and made acid with dilute hydrochloric acid. The mixture was extracted to completion (six or seven times) with 25-35 cc. portions of chloroform or about 35-50 cc. portions of ether, if emulsions formed. The chloroform extractions were filtered

through chloroform-wet cotton into a beaker and the solvent was evaporated to a small volume (4-3 cc.) on a steam bath with the aid of a brisk current of air, care being taken that active ebullition of the solvent did not take place. The solution was transferred with the aid of a little chloroform to a separator containing about 20 cc. of alkaline salt solution (0.5 N sodium hydroxide saturated with sodium chloride). The mixture was shaken thoroughly and the chloroform was allowed to separate as completely as possible. The chloroform was drawn off into a second separator containing about 3 cc. of 0.5 N sodium hydroxide. These were shaken well. The chloroform was allowed to separate completely and was then drawn off through chloroform-wet cotton into a tared dish. The extraction was repeated with three or four more 20 cc. portions of chloroform. The combined chloroform extracts were evaporated to dryness. A few treatments with anhydrous ether greatly assist in completely expelling the last traces of the chloroform solvent. The residue was dried at 90-100° C. for ten to fifteen minutes. Residue "A" may contain non-volatile aromatic compounds plus any neutral decomposition products.

The alkaline wash water was combined with the alkaline salt solution. A piece of litmus paper was placed in the separator and acidified with dilute hydrochloric acid. An excess of dry sodium bicarbonate was added in small portions. The phenobarbital was completely extracted with 20-25 cc. portions of chloroform. Each portion was filtered successively through chloroform-wet cotton into a tared dish. The solvent was evaporated with the usual precautions. The residue is usually syrupy due to traces of chloroform, which are tenaciously held until

superheating causes the chloroform to vaporize with explosive violence, thus causing losses by decrepitation. A few small additions of anhydrous ether materially shortens the time required to drive off the chloroform, minimize the loss by decrepitation, and leave dry, granular residues which possessed melting points very close to theory.

The sodium bicarbonate solution in the separator was then cautiously acidified with dilute hydrochloric acid. The mixture was extracted with three or four 20 cc. portions of chloroform or other appropriate solvent, filtered, and the solvent was evaporated as usual. The residue "C" was examined for decomposition or acidic drugs."

c. Method of van Itallie and Steenhauer (74)

The method of van Itallie and Steenhauer (74) is an immiscible-solvent method adapted for the quantitative determination of barbiturates in urine and organ extracts. In addition to the procedure which must be employed to remove extraneous matter, the only difference which is of interest in this discussion is the nature of the volatile solvent. These workers claimed that substituted barbituric acids are incompletely extracted by ether and that ethyl acetate eliminates this error by its greater solvent power. Veronal is more than twice as soluble in ethyl acetate as in ether. Mancani (75) described a procedure which was similar to that employed by van Itallie and Steenhauer (74).

d. Method of Glycart (76)

The barbituric acid is dissolved in a 2% solution of sodium hydroxide which is saturated with sodium chloride. The solution is shaken

with ether. The ether washing is discarded. The contents of the separator are then acidified with hydrochloric acid and extracted with a mixture of two parts of ethyl alcohol, one part of ether and seven parts of chloroform. The extract is evaporated to dryness and weighed. The method of Glycart (76), with slight modifications, was adopted as the official method of assay for barbital and phenobarbital by the Association of Official Agricultural Chemists (77). The official method employs a solvent consisting of two parts of ether and eight parts of chloroform.

The method of Glycart (76) was also employed, with slight modification, in the N. F. VI assays of Elixir of Phenobarbital, Elixir of Barbital, and Tablets of Phenobarbital (75) and in the U. S. P. XII assays of Barbital Tablets and Pentobarbital Tablets (71). In the U. S. P. XII (71) the assay of Elixir of Phenobarbital is changed to an assay consisting essentially of acidification with diluted hydrochloric acid and extraction with chloroform. In the N. F. VII (78) the assay of Elixir of Barbital was modified to the extent of using a solvent composed of one volume of ether and nine volumes of chloroform.

In addition to the above gravimetric methods, there are other types depending upon the formation of complex addition products, such as the complex products formed by the reaction of phenobarbital with mercuric iodide as reported by Montignie (79); the complex salts formed with iodine in an alkaline solution as reported by Bougault and Guillou (80); complex mercury salts as reported by Fleury (81); and the copper pyridinyl phenobarbital formed by the use of Zwikker's reagent (82). Bell (83) stated that most of the methods depending upon the formation of

complex addition products were unreliable, due probably to the inconsistent or unknown composition of the addition product.

2. Procedures Employing Indicators

Several indicators have been suggested for the direct titration of the replaceable hydrogen atom in the malonylurea group. The acid form of the barbiturate was dissolved in a solvent consisting wholly or in part of an organic substance.

Joensson (84) stated that veronal cannot be titrated directly with sodium hydroxide using phenolphthalein as an indicator. Explanation was made that the pH of a 0.1 molar solution of veronal sodium is pH 10.2 and that the pH of a 0.01 molar solution of the same compound is pH 9.7, whereas the color change of phenolphthalein occurs at pH 8.2. This difficulty was overcome by the use of an organic solvent such as acetone. The method of Joensson (84) consisted of dissolving the veronal in 20-25 cc. of acetone, adding 0.10 to 0.15 cc. of 1:2000 phenolphthalein solution, and titrating to a faint red color with 0.1 N sodium hydroxide. The method of Sol'ts (85) for the determination of luminal differed only in the use of alcohol as a solvent.

Von Mabitsch (86) employed thymolphthalein as an indicator in titrating veronal and luminal with sodium hydroxide because its transition limits of pH 9.3 to pH 10.6 encompass the pH of veronal sodium and phenobarbital sodium. Von Mabitsch (98) employed alcohol as the solvent. He obtained results which represented 99.77% of the samples taken. Gervay (87) also reported the use of thymolphthalein as an indicator for the titration of phenobarbital. The solvent in this case consisted of

methanol which was neutral to thymolphthalein. Gervay (87) suggested that alizarin yellow R, instead of thymolphthalein, be used as an indicator when titrating veronal. Belenits'ka (88) modified the method of von Babitsch (86) to the extent of using a solvent consisting of two parts of alcohol and one part of water.

Morin (89) titrated barbituric acids, which were dissolved in acetone, with a methanol solution of potassium hydroxide in the presence of thymol blue.

Emell (90) titrated barbital sodium in ether using iodeosin as the indicator.

3. Conductimetric Titration

Mrynakowski and Modrzejewski (91) stated that the application of conductimetric titration to the determination of weak acids and bases generally gave good results. They claimed to have determined barbital and phenobarbital with an average error of 0.2 to 0.5%. Substances insoluble in water but soluble in ethyl alcohol can be titrated in alcoholic solution. Better results were obtained, however, if the weak acids, after dissolving in excess of alkali, were titrated back with hydrochloric acid.

4. Procedures in which Undissociated Barbiturates are Formed.

(a) Mercury Salts

Ionescu-Matiu (92) and von Babitsch (86) developed procedures which are dependent upon the insolubility of the mercury salts of barbiturates in slightly acid media. The precipitant is prepared by dissolving

mercuric oxide in concentrated sulfuric acid and diluting with water. The precipitate formed by the addition of this reagent to the barbiturate solution is separated, washed, and dissolved in a mixture of sulfuric and nitric acids. The amount of mercury present is determined and from this the amount of barbiturate is calculated.

(b) Silver Salts

Budde (93) stated that the procedure of Stas-Otto (immiscible-solvent method) is troublesome and time consuming because of the repeated shaking out with ether.

Budde (93) claims that an alkalimetric determination of barbituric acid does not lead to satisfactory results. The probable presence of replaceable, enolic hydrogen atoms during titration with sodium hydroxide brings about only a slow change in hydrogen ion concentration, so that an exact end point can only be observed with difficulty and under special conditions.

Budde (93) developed a volumetric determination which is based on an observation of Zernik that mercuric oxide is dissolved by veronal in a presumably alkaline media. Budde (93) assumed that a mercuric chloride of known strength may be titrated into an alkaline veronal solution until an excess of mercury ions is brought into reaction with the alkali with separation of mercuric oxide. Since a standard solution of mercuric chloride is not official in the Deutsche Arzneibuch 6, Budde (93) proceeded to investigate whether or not silver nitrate solution acted in a similar way. It was found that in the presence of sodium hydroxide, 0.65 to 0.8 mols of silver nitrate was consumed by 1 mol of veronal upon the appearance of the first turbidity. Silver

nitrate is transformed by the sodium veronal to a silver barbiturate which is soluble in the alkaline media. Like mercury cyanide, the silver barbiturate is very difficultly dissociated, so that the excess of sodium hydroxide gradually and incompletely produces a separation of silver oxide.

Rupp and Poggendorf (94) reported that phenobarbital is precipitated by silver nitrate. The precipitate was found to be soluble in ammonia.

Viebock and Fuchs (95, 96) gave the equations as follows:

$$\underset{\text{(phenobarbital sodium)}}{C_{12}H_{11}O_3N_2Na} + NaOH + 2AgNO_3 \rightarrow C_{12}H_{10}O_3N_2Ag_2 + 2NaNO_3 + H_2O$$

$$\underset{\text{(barbital-Na)}}{C_8H_{11}O_3N_2Na} + NaOH + 2AgNO_3 \longrightarrow C_8H_{10}O_3N_2Ag_2 + 2NaNO_3 + H_2O$$

Tomski and Waller (97) gave the following as the course of the reaction:

$$2\ C_{12}H_{11}O_3N_2Na + 2AgNO_3 \longrightarrow \underset{\text{(a soluble, double compound)}}{C_{12}H_{11}O_3N_2Na\ C_{12}H_{10}O_3N_2Ag_2} + NaNO_3 + HNO_3$$

$$C_{12}H_{11}O_3N_2Na\ C_{12}H_{10}O_3N_2Ag_2 + 2AgNO_3 \rightarrow 2\ C_{12}H_{10}O_3N_2Ag_2 + NaNO_3 + HNO_3$$

Tomski and Waller (97) state that silver phenobarbital is insoluble, presumably meaning in acid or neutral media.

The first experiments of Budde (93) showed results which were 10 to 16% off. He thought that better results could be obtained by the use of weaker alkalies. However, later workers (95, 96, 96) indicate that sodium hydroxide may be used but the quantity must be regulated.

Budde (93) developed the following method:

About 0.2 to 0.3 Gm. of the sample are accurately weighed and dissolved in 30 cc. of water with 1 Gm. of anhydrous sodium carbonate. To the clear solution, 0.1 N silver nitrate is added until a distinct turbidity is produced, which remains for some time. Von Embitsch (86) found Budde's method (93) completely satisfactory.

The method employed by Kalinowski (98) involves a titration of an alcoholic or acetone solution of the barbituric acid in the presence of sodium hydroxide. To a solution of 0.2 to 0.3 Gm. of the substance to be analyzed in 20 to 25 cc. of acetone, or 30 to 35 cc. of ethyl alcohol, 15 to 20 cc. of a normal solution of sodium hydroxide and 20 to 30 cc. of water are added. This clear solution is titrated with 0.1 N solution of silver nitrate until a turbidity is obtained. Kalinowski (98) claimed the method to be accurate to 0.1%.

Viebock and Fuchs (95) explained that phenobarbital and barbital are monobasic toward alkalies and dibasic towards silver nitrate. If, to a solution of phenobarbital two equivalents of sodium hydroxide are added, then two equivalents of silver nitrate, the silver salt is precipitated and the solution is neutral.

$$C_{12}H_{11}O_3N_2Na + NaOH + 2AgNO_3 \longrightarrow C_{12}H_{10}O_3N_2Ag_2 + 2NaNO_3 + H_2O$$

When luminal or veronal is titrated with sodium hydroxide in the presence of excess silver nitrate, towards the end of the titration, silver hydroxide is formed which does not dissolve and which is easily concealed by the insoluble veronal-silver or luminal-silver. To overcome this difficulty, sodium salicylate is added. The latter replaces the excess

silver nitrate with silver salicylate. Silver salicylate has a moderate solubility, sufficient for a quantitative precipitation of veronal-silver or luminal-silver but on the other hand it hinders the formation of silver hydroxide.

The method of Viebock and Fuchs (95) for the determination of salts of barbiturates is as follows:

A. Determination of alkali.

Dissolve 0.2 Gm. of the compound in 25 cc. of water and titrate with 0.1 N sulfuric acid with methyl orange as the indicator.

This step is probably to regulate the quantity of alkali in the solution, inasmuch as barbiturate salts occur on the market with varying amounts of alkali (4). In the next step, a definite amount of alkali is added. This avoids the difficulty experienced by Budde (93) in his use of sodium hydroxide.

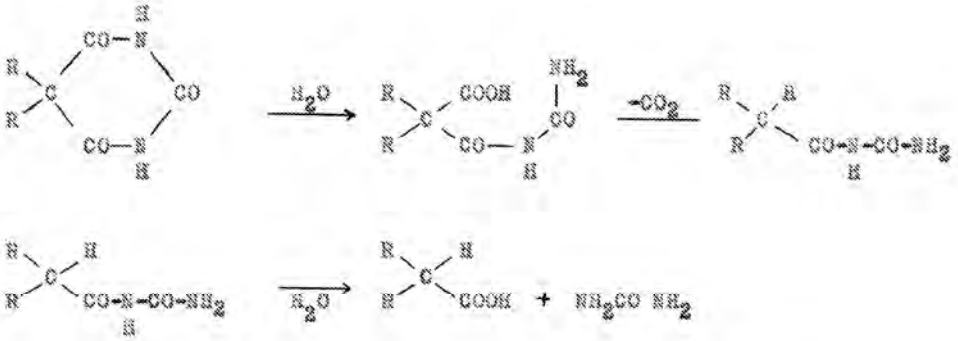
B. Determination of Phenobarbital and Barbital.

The solution from part A is freed of carbonic acid by heating. A measured quantity of 0.1 N carbonate-free alkali is added. For 7.5 cc. of acid used in the first titration, 14.0 to 14.5 cc. of alkali are used in the second titration (generally 0.5 to 1.0 cc. less than double the quantity of acid used in the first titration). About 25 cc. of 0.1 N silver nitrate is added (5 to 10 cc. more than double the quantity used in the first titration). After a further addition of 0.5 to 1.0 Gm. of sodium salicylate, the solution is titrated with 0.1 N alkali, with phenolphthalein as the indicator. With an absolutely pure preparation, the quantity of alkali used in B must be exactly twice the quantity of acid used in A.

Fuchs (99) also employed the above method to assay diethyl barbituric acid by titrating the free acid, which was dissolved in alcohol, with alkali in the presence of thymolphthalein. The second part of the assay outlined above was unchanged.

It would seem from the work of Budde (93), Viebock and Fuchs (95, 96), and Tomski and Waller (97) that the barbituric acids are dibasic towards silver nitrate in neutral media and monbasic towards silver nitrate in alkaline media.

Madsen (3) and Nielsen (15) followed the course of hydrolysis of barbiturates from the amount of dialkylacetylurea and carbon dioxide formed. Schlemmer and Torber (30) state that this method is incomplete since hydrolysis of dialkylacetylurea is assumed to proceed further to produce diethylacetic acid and urea, as shown by the equations which follow.



$$NH_2CONH_2 \xrightarrow{H_2O} 2\,NH_3 + CO_2$$

The values of percentage decomposition obtained by Madsen (3) are lower than those obtained when Schlemmer and Torber (30) employed Budde's procedure (93). This provides evidence for the criticism of Schlemmer and

Torber (30), mentioned above.

Bell's (83) application of Kalinowski's modification (98) of the Budde method (93) to the analysis of theobromine and phenobarbital mixtures is as follows:

The sample containing theobromine and phenobarbital is boiled with 2 N sulfuric acid. The mixture is cooled to 40° C. and transferred to a separatory funnel. The mixture is shaken out with alcohol-free ether. The extracts are filtered through cotton. The combined ether extracts are evaporated. The residue is dissolved in exactly 100 cc. of a solvent consisting of 50 cc. of ethyl alcohol, 10 cc. of water and 40 cc. of 1.0 N sodium hydroxide. The solution is filtered and the first quarter is discarded. The aliquot three-quarters remaining is titrated with 0.1 N silver nitrate until a distinct turbidity is evident. The silver nitrate must be added very slowly and toward the end point must be added drop by drop, shaking vigorously after the addition of each drop. The immediate turbidity caused by the addition of each drop must be cleared by shaking before the addition of the next drop. The end point is characterized by a persistent turbidity of the solution even after vigorous shaking. The titrated solution at this point begins to change rapidly to a brownish-black color due to the separation of silver oxide.

Bell (83) states that the alkalinity of the solution of extracted phenobarbital should not be varied appreciably from the concentration mentioned because the sharpness of the end point is influenced by this factor.

Schulek and Rozsa (100, 101) devised an argentometric assay of

barbiturates with potassium chromate as the indicator. The barbiturate (0.1-0.18 Gm.) was dissolved by boiling in 25 cc. of a 5% borax solution. Potassium chromate (1 cc. of a 10% solution) was added. Silver nitrate, 0.1 N, was introduced into the hot mixture until the yellowish-green, turbid liquid attained a reddish color, which persisted even after boiling. Since Schulek and Rozsa (100, 101) stated that 1 cc. of 0.1 N silver nitrate is equivalent to 0.250 mg. of barbital, it may be concluded that both hydrogen atoms attached to the nitrogen atoms were replaced.

5. Colorimetric Methods

(a) Method of Zwikker (102).

Barbiturates react with cobalt chlorids in anhydrous media to give a red color. Addition of barium oxide converts this to blue. A red dibarbitalocobaltodiammine has been isolated by Zwikker (103).

(b) Method of Oettel (105).

The sample is extracted with chloroform and filtered. The solution is diluted as necessary. Two (2) cc. of the sample is transferred to each of three test tubes. Then 0.05 cc., 0.1 cc. and 0.15 cc. of 0.2% cobalt acetate solution in absolute methanol are added. After shaking, to the same respective tubes 0.05 cc., 0.1 cc. and 0.15 cc. of a 0.2% solution of lithium hydroxide in absolute methanol are added. These volumes must be accurately measured.

If all three tubes or only the last two give an intense blue color, the chloroform solution contains more than 0.1 mg. per cc. of a barbituric acid derivative. If the test is positive in the first two tubes

and negative or disappears within a minute in the last, the chloroform extract contains about 0.05 mg. of a barbituric acid derivative. If only the first is positive, the chloroform extract contains about 0.025 mg. of a barbituric acid derivative. With practice, fair accuracy can be attained. By comparison with standards greater accuracy should be possible.

If all samples are positive or the two most concentrated are positive, dilute the chloroform extract. A large excess of barbituric acid derivatives can give a negative reaction or a gray color in all of the tubes. In that case, dilute the extract with chloroform until the blue color is obtained.

(c) Method of Koppanyi et al. (106).

The colorimetric estimation is based on the blue color produced by cobalt acetate in alkaline solution. When the test is performed with 1% cobalt acetate and 1% barium hydroxide in absolute methanol, it is sensitive to one part of barbital in 10,000 parts of solution. When the test is performed with 0.2% cobalt acetate and 0.2% lithium hydroxide in absolute methanol, it is directly sensitive to one part of barbital in 100,000 parts of solution and 1 in 2,000,000 after concentration of the chloroform solution twenty times. The accuracy of this method is limited to about 8%.

III EXPERIMENTAL PART

A. SCOPE OF INVESTIGATION

The products formed during the decomposition of barbiturates and the manner in which they are produced constituted the research of several workers, namely, Steenhauer (2), Madsen (3), Bailey (4), Nielsen (10, 11, 13) and Aspelund and his coworkers (7, 8, 9). From the agreement in the results which were reported by these men, the nature of the decomposition appeared to be amply explained. However, it was felt that an investigation of a number of factors in the deterioration of phenobarbital and phenobarbital sodium, together with research on several methods of retardation, would add to the existing knowledge concerning these phenomena.

Evaluation of several reported methods of assay were made in an attempt to ascertain their applicability to such a study. Methods employing immiscible solvents and methods employing silver nitrate were studied. Suitable methods of determining the percentage moisture were sought inasmuch as its accurate determination was essential in preparing solutions and in checking the accuracy of the assay used in the experiments on deterioration and stabilization. The experiments on the deterioration of Phenobarbital Sodium U. S. P. XI in various concentrations included the determination of volume of precipitate, change in pH, change in refractive index, and percentage loss of barbiturate, when the solution was subjected to heating at various temperatures for various periods of time. The relation between pH and deterioration was studied by making use of sodium carbonate and phosphate buffers to

regulate the pH. A number of suggested stabilising agents were subjected to tests and assays to determine their influence on the retardation of deterioration.

B. ANALYTICAL METHODS AND CONTROL

1. Materials used

The phenobarbital was purchased from the Mallinckrodt Chemical works. It was labeled "Phenobarbital U. S. P. XI, Control MNCl." Data given elsewhere in the discussion showed that the drug conformed to the standards of the U. S. P. XI and U. S. P. XII as regards moisture content and melting point. The phenobarbital sodium was likewise purchased from the Mallinckrodt Chemical Works. Two lots were obtained. The labels read "Phenobarbital Sodium U. S. P. XI." The control designations were MKM and MLM1. Data given subsequently revealed that these lots conformed to the standards of the U. S. P. XI and U. S. P. XII as regards moisture content and purity rubric. The particular lot which was used in an experiment is mentioned in the review of the experiment. The source of the other chemicals employed is mentioned in the discussion of the experiments concerned.

2. Determination of Moisture

Unless otherwise stated, the samples of phenobarbital sodium which were directly used in an experiment were of known moisture content. Quantitative determination of phenobarbital sodium was calculated on a moisture-free basis, i.e., a lot of drug whose moisture content had previously been determined on separate samples. As subsequently discussed, it was deemed advisable to dry the samples to constant weight

at temperatures slightly above 100° C. The values obtained by drying samples in a vacuum desiccator approached those obtained by drying samples at the latter temperature. The term, dried to constant weight, was interpreted to mean that two consecutive weighings do not differ by more than 0.1 per cent when the second weighing is made after an additional hour of drying. Samples not exceeding 0.8 Gm. were taken for the moisture determinations which were made at approximately 100° C. This was done in order to assure complete drying by exposing a thin layer of drug to the surrounding air in the oven. A Freas Thermo Electric Oven was the drying chamber employed.

3. pH Determination

The Beckman pH Meter, laboratory model G, was employed in all the determinations of pH. For measurements above pH 9 the Beckman, high-pH, No. 1190-E, glass electrode was used. This electrode is intended for measurements made on alkaline solutions of high pH at room temperature. In highly alkaline solutions this electrode does not attain equilibrium instantly but may indicate a gradually increasing pH for five minutes or more at approximately 25° C. The time required for attainment of equilibrium was shortened by keeping the tip of the glass electrode immersed in pH 10 buffer, while not in use. The asymmetry potential also tends to change slightly during measurement, acting to reduce the pH reading. The usual result is to give a pH curve against time that reaches a maximum in about five minutes followed by a slow decrease in reading. Standardization of the electrodes and meter against a pH 10 buffer was made after every two readings, frequently after each reading When the glass electrode attained equilibrium slowly, pH readings were

made at two or three minute intervals until no further upward drift was noted. The buffer standardization was then made. If the latter reading differed from the pH value of the buffer, the difference between the two was either subtracted from or added to the last reading of the sample, as required to make the proper correction. In the event the equilibrium was rapidly attained, the maximum pH reading was taken as correct for the sample.

The Beckman 015 type glass electrode was used for measurements below pH 9.

4. Determination of Volume of Precipitate

Inasmuch as the amount of precipitate formed in heated solutions of phenobarbital and phenobarbital sodium became greater with increase in temperature, a method of approximating the volume of precipitate was devised. With shaking, the contents of the bottle, which had been subjected to heating, were transferred to a beaker. While the mixture was rapidly stirred by means of an electric stirrer, a convenient volume, usually 10 cc., was withdrawn by means of a pipette. The contents of the pipette were placed in a graduated centrifuge tube. If the mixture could not be drawn into a pipette, the contents of the bottle were introduced with shaking into a centrifuge tube. In either case the tube was centrifuged at approximately 1200 r.p.m. for five minutes as timed by a stopwatch. Time was taken the moment the current was turned on and the moment it was shut off. It took approximately twenty seconds for the centrifuge to cease revolving.

It is not purported that this determination should be taken as quantitative. However, the method is considered more satisfactory than

the use of such words as "large," "moderate," or "small" in describing the quantity of precipitate.

5. Use of the Autoclave

To attain the higher temperatures to which some of the solutions were subjected in this investigation, the bottles containing the solutions were exposed to saturated steam under pressure. The apparatus employed was a "Peerless" pressure cooker. The following procedure was resorted to in order to gauge the time during which the solution was exposed to a certain temperature: the autoclave was heated until steam was issuing strongly from the petcock. The lid was removed and the bottles were introduced into the chamber. The lid was immediately replaced and clamped down. The autoclave was rapidly heated by several Meker burners in order to quickly saturate the space in the chamber with steam. As soon as all the air was replaced with steam, the petcock was closed, causing the pressure to rise rapidly. When the desired pressure was attained, time was taken by means of a stopwatch. The Meker burners were then shut off and the pressure was maintained at a constant level by means of a low bunsen flame. The variance of temperature was $\pm 1^{\circ}$ C. When the period of time during which the solution was supposed to have been exposed to heating had elapsed, the petcock was opened completely to permit rapid escape of steam. The autoclave was quickly opened and the bottles were immediately inserted in a mixture of ice and water to rapidly cool the solutions.

C. A COMPARISON OF METHODS OF DRYING PHENOBARBITAL SODIUM PREPARATORY TO ASSAY

The purpose of comparing various methods of drying phenobarbital sodium to constant weight was to detect the presence of decomposition which might occur during drying. Phenobarbital sodium absorbs considerable moisture from its surroundings due to its hygroscopic nature. Inasmuch as solutions of phenobarbital sodium undergo hydrolytic decomposition, there is a possibility that the compound may suffer hydrolysis when heated in the solid state, particularly if it contains an appreciable quantity of moisture. A method of drying to constant weight without producing decomposition was necessary in determining the dry weight of samples which were used in the study of various methods of assay of phenobarbital sodium and in the study of decomposition of solutions of the compound. In the latter case it was essential to prepare solutions of definite concentration from samples whose dry weight was known. Not only could the concentration of solutions be determined from the amount of dry sample present in a unit volume but the consistency of the method of assay and the per cent of phenobarbital sodium present in the dry sample could be calculated.

The drug employed was purchased from the Mallinckrodt Chemical Works. It was labeled Phenobarbital Sodium U. S. P. XI and it bore the control number KMl. Samples were heated to constant weight in an oven at 105° C. These samples were then heated to constant weight at 140° C. to determine whether or not an additional loss in weight would result. Additional samples were heated at 140° C. Other samples were spread out in petri dishes and kept in an evacuated dessicator

for one month. Samples which had been heated were assayed by the Budde method to detect the presence of decomposition.

The U. S. P. XI limits the amount of moisture in phenobarbital sodium to seven per cent as determined by heating the salt at 140° C. for six hours. It was stated by van Leent (27) that heating phenobarbital sodium at 120° C. produced a product which gradually became incompletely soluble. No difficulty was experienced during this investigation in dissolving samples which had been dried to constant weight at 142 (± 1)° C. during a period of ten hours.

TABLE 14

DETERMINATION OF MOISTURE IN PHENOBARBITAL SODIUM U. S. P. XI

PART A. LOSS IN WEIGHT ON HEATING PHENOBARBITAL SODIUM U. S. P. XI TO CONSTANT WEIGHT AT 101 (±2)° C.

Weight in Gm.	Duration of Heating in Hours	Loss of Weight in Gm.	Percentage Loss of Weight
(1) 2.6459	12	0.0459	1.7
(2) 3.3706	12	0.0590	1.7
(3) 1.0490	8	0.0191	1.8
(4) 1.1427	8	0.0198	1.7
(5) 1.0771	8	0.0188	1.8
(6) 0.8437	8	0.0147	1.7
		Average	1.7

PART B. ADDITIONAL LOSS IN WEIGHT ON HEATING THE ABOVE SAMPLES TO CONSTANT WEIGHT AT 141 (±1)° C.*

Weight in Gm.	Loss of Weight in Gm.	Percentage Loss of Weight	Total Loss of Weight %
(1) 2.6000	0.0443	1.7	3.4
(2) 3.3126	0.0566	1.7	3.4
(3) 1.0699	0.0187	1.7	3.5
(4) 1.1288	0.0192	1.7	3.4
(5) 1.0583	0.0182	1.7	3.4
(6) 0.8290	0.0140	1.7	3.4
	Average	1.7	3.4

*Six hours of heating were required to attain constant weight

TABLE 16

LOSS IN WEIGHT ON HEATING PHENOBARBITAL-SODIUM U. S. P. XI TO CONSTANT WEIGHT AT 141 (±1)° C.*

Weight in Gm.	Loss of Weight in Gm.	Percentage Loss of Weight
(1) 1.1223	0.0380	3.4
(2) 1.0635	0.0366	3.4
(3) 1.0041	0.0341	3.4
(4) 1.0206	0.0351	3.4
(5) 1.1007	0.0377	3.4
	Average	3.4

*Seven hours of heating were required to attain constant weight.

TABLE 18

LOSS IN WEIGHT ON VACUUM DESICCATION OF PHENOBARBITAL-SODIUM U. S. P. XI*

Weight in Gm.	Loss of Weight in Gm.	Percentage Loss of Weight
(1) 11.5406	0.1700	1.5
(2) 11.6715	0.1676	1.4
(3) 13.0285	0.1901	1.5
	Average	1.5

*The samples were kept for 31 days in a vacuum desiccator evacuated to approximately 0.5 mm Hg.

In the three preceding tables it is seen that the lot of Phenobarbital Sodium U. S. P. XI employed in this study decreased 1.7 per cent in weight on drying to constant weight at 101 (±2)° C. A further 1.7 per cent loss was undergone upon heating the same samples to constant weight at 141 (±1)° C. The total loss in weight was 3.4 per cent, which figure was likewise obtained on drying samples exclusively at 141 (±1)° C. On drying samples in an evacuated dessicator for one month, the loss in weight was 1.8 per cent or a value approaching that obtained on drying phenobarbital sodium to constant weight at 101 (±2)° C. These results indicated either incomplete drying at approximately 100° C. or the presence of decomposition at approximately 140° C.

To determine whether or not decomposition occurred during the drying of samples at the aforesto mentioned temperatures, dried and undried samples were assayed by the Budde method.

TABLE 17

BUDDE ASSAYS OF DRIED AND UNDRIED PHENOBARBITAL SODIUM U. S. P. XI

PART A

Sample Before Drying Gm.	Sample After Drying at 103 (±1)° C.	Loss in Weight Per Cent	0.1N $AgNO_3$ cc.	$C_{12}H_{11}O_3N_2Na$ Gm.	Per Cent of Undried Sample
0.4586	0.4432	3.4	17.06	0.4332	94.4
0.6120	0.5913	3.4	22.56	0.5728	94.4
0.3506	0.3387	3.4	12.93	0.3286	93.7
	Av.	3.4		Av.	94.2

PART B

Sample Before Drying Gm.	Sample After Drying at 142 (±1)° C.	Loss in Weight Per Cent	0.1N $AgNO_3$ cc.	$C_{12}H_{11}O_3N_2Na$ Gm.	Per Cent of Undried Sample
0.4558	0.4337	4.9	16.78	0.4287	94.1
0.4463	0.4246	4.9	16.60	0.4193	93.9
0.5648	0.5370	4.9	20.85	0.5293	93.8
	Av.	4.9		Av.	93.9

PART C

Undried Sample Gm.	0.1N $AgNO_3$ cc.	$C_{12}H_{11}O_3N_2Na$ Gm.	Per Cent of Undried Sample
0.2821	10.40	0.2643	93.7
0.2634	9.74	0.2474	93.9
0.3211	11.79	0.2996	93.3
		Av.	93.6

On the basis of undried sample, 94 per cent phenobarbital sodium was present in samples of Phenobarbital Sodium U. S. P. XI before and after drying at 108 (±1)° C. and at 142 (±1)° C. Therefore, the presence of decomposition in samples heated at approximately 100° C. and approximately 140° C. was not shown by the Budde Assay.

Throughout this investigation undried samples were used except where otherwise indicated. The equivalent weight of dry sample, upon which the percentages were usually based in this study, was calculated from loss of weight sustained on heating other samples to constant weight at temperatures slightly above 100° C. unless otherwise indicated. This loss in weight at approximately 100° C. was employed in preference to that sustained at approximately 140° C. because of possible decomposition which may occur at the higher temperature.

D. EVALUATION OF REPORTED METHODS OF ASSAY

Several methods of determining the percentage deterioration of barbiturate solutions have been reported. Nielsen (13) and Madsen (8) followed the process quantitatively by measuring the carbon dioxide and dialkylacetylurea formed. Schlemmer and Förber (30) found that the results obtained by such a method were too low because of the existence of partial decomposition of dialkylacetylurea. Tomski and Waller (24) determined the amount of undecomposed barbiturate by titration with a standard solution of silver nitrate in the presence of sodium carbonate. Rotondaro (72), Glycart (70) and Aspelund and Skoglund (7) separated various decomposition products from one another at various pH levels. In addition other methods were reported but their

use was mentioned in connection with assays of undecomposed barbiturates. The methods employing an immiscible solvent at a single pH level were considered unsatisfactory for the investigation. The methods in which the alkalinity or acidity of the barbiturate derivative was measured by simple titrating with a standard acid or alkali were not applicable because of the presence of alkaline decomposition products in deteriorated solutions. Colorimetric methods were not investigated because of their reported inaccuracies.

The method of Rotondaro was chosen as a method worthy of investigation because a quantitative separation of the undecomposed barbiturate was claimed. The U. S. P. XI method (70) was studied for the sake of comparison. The Budde (93), Viebock and Fuchs (96, 96), and Schulek and Rozsa (100, 101) methods exemplified the several methods employing silver nitrate. The possibility of applying the von Babitsch titration (86) to the determination of the quantity of the free barbituric acid in the residues recovered in some immiscible-solvent methods was investigated.

1. Comparison of U. S. P. XI and Rotondaro Assays of Phenobarbital Sodium U. S. P. XI

The purpose of the experiment was to compare the U. S. P. XI assay (70) with the Rotondaro assay (72) and a modified form of the Rotondaro assay.

The U. S. P. XI (70) assay of phenobarbital sodium is given as follows: "Dissolve about 0.5 Gm. of Soluble Phenobarbital, dried at

140° C. for six hours, and accurately weighed, in 50 cc. of distilled water in a separator. Add 10 cc. of diluted hydrochloric acid, and completely extract the liberated phenobarbital with successive portions of 25 cc. each of ether. Evaporate the combined ethereal extracts in a tared dish, and dry the residue to constant weight at 100° C. The weight of phenobarbital so obtained is not less than 90.4 and not more than 91.4 per cent of the weight of the Soluble Phenobarbital taken for the assay." The assay was modified to the extent of using undried samples of known moisture content. This was done to avoid the possibility of incurring decomposition during the drying of the sample.

The Rotondaro method (72) was discussed in the review of the literature. The method was modified in some of the assays for the purpose of simplification and elimination of details which did not have to be retained in following the decomposition of barbiturate solutions. Instead of acidifying at the start, the solution of the barbiturate was treated with a proportional amount of normal sodium hydroxide equivalent to the amount of alkali which Rotondaro (72) used to extract the acidic compounds from the chloroform extracts containing both the barbiturate and the decomposition products. The volume of solution prepared or taken for the assay was such that the dilution with the normal solution of sodium hydroxide resulted in a concentration of alkali approximating a 0.5 N sodium hydroxide. At the same time the ratio between the total amount of alkali and barbiturate was maintained as found in the original assay. The alkaline solution was extracted with chloroform to remove the neutral decomposition product, phenylethylacetylurea. The net result of the modification was to extract the

neutral decomposition product from an alkaline solution instead of leaving this product behind in the chloroform on extracting the latter, which contained both the barbiturate and the decomposition products, by means of an alkaline solution. The remainder of the assay was unaltered. Residue I indicates phenylethylacetylurea, residue II indicates phenobarbital, and residue III indicates the acidic decomposition products, such as phenylethylmalonuric acid and phenylethylacetic acid. The shaking device designed by Mittelstaedt, Horn, and Kaufman (107) was employed to perform the shaking-out with immiscible solvent as required in the experiments subsequently mentioned. Each period of shaking-out was not less than ten minutes in duration.

All the residues obtained by the Rotondaro procedure (72) were dried to constant weight at temperatures slightly above 100° C., successive weighings being made at fifteen minute intervals.

Samples of Phenobarbital Sodium U. S. P. XI, possessing the Mallinckrodt control designation KLM1, were employed in this study of evaluation of assays. The U. S. P. XI (70), Rotondaro (72), and modified Rotondaro assays were performed on samples of known moisture content. In addition a number of modified Rotondaro assays of samples which had been dried at 141 (±1)° C. were conducted. The results are summarized in the tables which follow.

TABLE 18

U. S. P. XI ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI*

Day	Calculated Sample Gm.	Phenobarbital Extracted Gm.	%	Calculated $C_{12}H_{11}O_3N_2Na$ Gm.	%
(1)	0.7157	0.646	90.4	0.707	99.0
(2)	0.5149	0.468	90.8	0.512	99.4
(3)	0.6573	0.596	90.7	0.655	99.3

*Undried samples were employed. The percentages were calculated on a moisture-free basis. Separate samples, which were dried at 102 (±1)° C., had undergone a loss in weight of 2.2%.

TABLE 19

ROTONDARO ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI*

PART A

Calculated Dry Sample Gm.	Residue I Gm.	%	Residue II** Gm.	%	Residue III Gm.	%
(1) 0.3411	0.004	1.2	0.263	77.0	0.033	9.6
(2) 0.3179	0.004	1.3	0.253	79.7	0.023	7.3
(3) 0.3160	0.005	1.6	0.250	79.2	0.023	7.3

PART B

Calculated $C_{12}H_{11}O_3N_2Na$ Gm.	%	Sum of the Three Residues Gm.	%	Sum of I, III, with II Calculated as $C_{12}H_{11}O_3N_2Na$ Gm.	%
(1) 0.287	84.3	0.299	87.7	0.324	95.0
(2) 0.277	87.2	0.280	88.2	0.304	95.7
(3) 0.274	86.7	0.278	88.1	0.302	95.6

*Undried samples were employed. The percentages were calculated on a moisture-free basis. Separate samples, which were dried at 102 (±1)° C., had undergone a loss in weight of 2.2%.

**The quantity of phenobarbital constituting Residue II was determined by the von Babitsch titration (86) which is discussed in the section entitled "Evaluation of Reported Methods of Assay."

TABLE 20

MODIFIED ROPONDARO ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI*

PART A

Calculated Dry Sample Gm.	Residue I Gm.	%	Residue II Gm.	%	Residue III Gm.	%
(1) 0.4951	0.006	1.0	0.390	78.7	0.046	9.2
(2) 0.4907	0.006	1.1	0.381	78.5	0.017	3.5
(3) 0.6004	0.010	1.9	0.468	78.5	0.026	5.2

PART B

Calculated $C_{12}H_{11}O_3N_2Na$ Gm.	%	Sum of the Three Residues Gm.	%	Sum of I, III, with II Calculated as $C_{12}H_{11}O_3N_2Na$ Gm.	%
(1) 0.427	86.2	0.440	88.9	0.477	94.2
(2) 0.396	80.4	0.383	78.1	0.418	85.1
(3) 0.403	80.5	0.404	80.7	0.439	87.6

*Undried samples were employed. The percentages were calculated on a moisture-free basis. Separate samples, which were dried at 100 (±1)° C., had undergone a loss in weight of 2.2%.

TABLE 21

MODIFIED ROSENDAHL ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI WHICH HAD BEEN DRIED TO CONSTANT WEIGHT AT 141 (±1)° C.*

PART A

Dry Sample	Residue I		Residue II		Residue III	
Gm.	Gm.	%	Gm.	%	Gm.	%
(1) 0.8700	0.000	0.0	0.824	85.0	0.063	6.5
(2) 0.9855	0.003	0.3	0.832	84.5	0.060	6.1
(3) 1.0645	-	-	0.907	83.7	-	-
(4) 1.0269	-	-	0.877	85.4	-	-

PART B

Calculated $C_{12}H_{11}O_3N_2Na$		Sum of the three residues		Sum of I, III, with II calculated as $C_{12}H_{11}O_3N_2Na$	
Gm.	%	Gm.	%	Gm.	%
(1) 0.903	93.0	0.888	91.5	0.965	99.6
(2) 0.911	92.5	0.895	90.9	0.974	99.8
(3) 0.993	91.6	-	-	-	-
(4) 0.981	93.5	-	-	-	-

*The average percentage loss in weight on heating these samples was 3.4%.

The phenobarbital sodium employed in these assays conformed to the requirements of the purity rubric of the U. S. P. XI inasmuch as 91% phenobarbital was extracted during the performance of the U. S. P. XI assay. By means of the Rotondaro method (72), 78.6% phenobarbital was extracted. By means of the modified Rotondaro method, 78.2% phenobarbital was extracted. This indicated the existence of several possibilities: (a) the original sample was partially decomposed; (b) decomposition took place during the modified and unmodified Rotondaro assays; and (c) incomplete extraction, and/or failure of the assay to separate the decomposition products quantitatively without admixture. The presence of the third possibility was further indicated by the sum of the three residues obtained in the modified Rotondaro assay of samples dried at 141 (±1)° C. This sum is comparable to the residues obtained in the U. S. P. XI assays inasmuch as the latter does not separate the decomposition products. In the latter set of Rotondaro assays, the sum of the three residues came to 91.2%. This figure, representing the best results obtained, is within the U. S. P. XI tolerance of 90.4-91.4%; in the assays given in Tables 19 and 20, the sum of the three residues was lower, showing inefficient extraction.

2. Comparison of Modified Rotondaro Assays of Phenobarbital and Phenobarbital Sodium

In an attempt to learn why the residues of phenobarbital had been lower in the Rotondaro assays, both modified and unmodified, than in the U. S. P. XI assays performed on phenobarbital sodium, determinations of Phenobarbital U. S. P. XI and phenobarbital, recovered from previous

Rotondaro assays, were conducted.

The Phenobarbital U. S. P. XI which was used in this experiment had a melting point of 175° C. The melting points of the individual residues, which made up the mixture employed in one set of assays, varied from 174.5° C. to 181.0° C. The U. S. P. XII (71) gives the melting point of phenobarbital as 174-178° C.

In addition the modified Rotondaro assay of Phenobarbital Sodium U. S. P. XI was repeated on the same lot of drug used in the foregoing experiment, namely, Mallinckrodt XLMI. The number of treatments with chloroform to extract each residue was increased from the number originally employed by Rotondaro (72), namely, six or seven, to twelve, in an attempt to increase the efficiency of extraction. Each period of shaking-out with the mechanical shaker was not less than ten minutes in duration.

TABLE 22

MODIFIED ROTONDARO ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI*

Sample Number	1	2	3
Calculated Dry Sample Gm.	1.1545	1.1257	1.3352
Residue I Gm.	0.012	0.037	0.028
per cent	1.0	3.3	2.1
Residue II Gm.	0.810	0.800	0.949
per cent	70.2	71.1	71.1
Residue III Gm.	0.165	0.141	0.183
per cent	14.1	12.5	13.7
Residue II calculated as $C_{12}H_{11}O_3N_2Na$ Gm.	0.868	0.876	1.040
per cent	76.9	77.8	77.9
Sum of the three residues with II calculated as $C_{12}H_{11}O_3N_2Na$ Gm.	1.062	1.054	1.280
per cent	92.0	93.6	93.6

*Undried samples were employed. The percentages were calculated on a moisture-free basis. Separate samples, which were dried to constant weight at 106 (±1)° C., had undergone a loss in weight of 4.2%.

TABLE 23

MODIFIED ROTONDARO ASSAY OF PHENOBARBITAL U. S. P. XI*

Sample Number	1	2	3
Calculated Dry Sample Gm.	1.1600	1.2826	1.3835
Residue I Gm.	0.007	0.013	0.013
per cent	0.6	1.0	0.9
Residue II Gm.	0.876	0.978	1.078
per cent	75.8	77.4	77.9
Residue III Gm.	0.180	0.166	0.162
per cent	15.6	13.1	11.7
Sum of the three residues Gm.	1.063	1.156	1.253
per cent	91.7	91.5	90.5

*Undried samples were employed. The percentages were calculated on a moisture-free basis. Separate samples, which were dried to constant weight at 108 (±1)° C., had undergone a loss in weight of 0.2%.

TABLE 24

MODIFIED ROTONDARO ASSAY OF PHENOBARBITAL*

Sample Number		1	2	3
Sample Weight	Gm.	0.7340	0.7025	0.6568
Residue I	Gm.	0.205	0.018	0.021
	per cent	3.5	2.5	3.2
Residue II	Gm.	0.531	0.538	0.493
	per cent	72.2	76.6	75.0
Residue III	Gm.	0.121	0.107	0.076
	per cent	16.4	15.2	11.6
Sum of the three residues	Gm.	0.676	0.662	0.590
	per cent	92.1	94.3	89.8

*The phenobarbital employed in these assays represented a mixture of dry phenobarbital residues obtained from previous Rotondaro assays. The melting points of the individual residues varied from 174.8° C. to 181.0° C.

By means of the modified Rotondaro assay, 70.8% phenobarbital was extracted from samples of Phenobarbital Sodium U. S. P. XI. The sum of the three residues averaged 86.4%. These results further substantiated the conclusions reached in the last experiment.

Modified Rotondaro assays of Phenobarbital U. S. P. XI yielded 77.0% recovery of phenobarbital. Modified Rotondaro assays of phenobarbital which had been previously obtained in other Rotondaro assays yielded 78.8% recovery of phenobarbital.

Although the number of treatments with chloroform to extract each residue was increased, the sum of the three residues obtained in the modified Rotondaro assays of Phenobarbital Sodium U. S. P. XI was below the U. S. P. XI tolerance of phenobarbital extracted. Moreover, the average of the sum of the three residues obtained in the modified Rotondaro assays of Phenobarbital U. S. P. XI and phenobarbital which had been previously obtained in the other Rotondaro assays, was only 91.7%. When 78.8% phenobarbital remains undecomposed, the loss of carbon dioxide from phenobarbital amounts to 2.9% of the phenobarbital taken. The difference between the sum of the three residues given in Table 24 and 100% recovery is only partially accounted for by the loss of carbon dioxide.

From the results obtained in this experiment, the existence of several possibilities in both the modified and unmodified Rotondaro assays is indicated: (a) occurrence of decomposition during the assay, (b) incomplete extraction, and (c) failure of the assay to separate the decomposition products quantitatively without admixture. Rotondaro (72) had claimed that 98% of the phenobarbital actually present

in a sample could be recovered by the use of his assay.

From the results obtained, it was concluded that the Rotondaro method, modified or unmodified, was not suitable for the quantitative determination of deterioration occurring in solutions of phenobarbital and phenobarbital sodium.

3. A Study of Assays Employing Silver Nitrate

The methods of Budde (93), Viehöck and Fuchs (95, 96), and Schulek and Rozsa (100, 101) exemplify the reported methods of assay employing silver nitrate for the determination of barbiturates. A comparison of results obtained by applying these assays on phenobarbital sodium constituted the purpose of this experiment. In addition an insight into the chemistry of the Budde assay (93) was sought.

The Budde assay (93) was conducted in the following way: About 0.2 to 0.3 Gm. of phenobarbital sodium was accurately weighed and dissolved in 30 cc. of water, together with 1 Gm. of anhydrous sodium carbonate. Andydrous sodium carbonate of analytical reagent quality was employed throughout the investigation whenever this compound was called for. The solution was prepared in a 50 cc. beaker which was supported by a universal clamp, attached to a ring stand at a convenient height. An electric stirrer was used to stir the solution vigorously throughout the titration. By means of a microburet, 0.1 N silver nitrate was introduced drop by drop. The dropping was regulated so that the turbidity produced by a drop of silver nitrate had disappeared before the introduction of the next drop. It was found that a time interval of one to

two seconds was sufficient to permit clearance of the solution. A stop-watch was employed to assist in maintaining a constant rate of introduction of the silver nitrate. The rate was maintained at one drop every one or two seconds. The apparatus was set up in a darkened room. A strong beam of light emitted by a microprojector was directed through the solution. The solution was viewed at a right angle to the ray of light. In this manner a faint turbidity was easily detected which would not be observable in diffused light. The appearance of a distinct turbidity which persisted for at least one minute constituted the end point. Budde (93) explained that a silver compound of the barbiturate was formed which was soluble in this media. Budde (93) stated that the silver barbiturate was very difficultly dissociated. This elucidated why the silver nitrate reacted with the barbiturate instead of reacting with the sodium carbonate. Silver carbonate and silver oxide are well dissociated. The silver ions are removed from the field of reaction by the formation of a poorly dissociated organic compound, namely, the silver barbiturate derivative.

In order to learn more concerning the assay, several tests were carried out. To show that silver phenobarbital is only slightly ionized in an alkaline media, a solution of Phenobarbital Sodium U. S. P. XI, was treated under conditions existing in the Budde assay. To 20 cc. of a 1.3% solution of Phenobarbital Sodium U. S. P. XI, 5 cc. of water, 0.03 Gm. of anhydrous sodium carbonate, and 5 cc. of 0.1 N silver nitrate were added. The resulting solution was clear as observed with the aid of a strong beam of light. Upon the addition of 1 cc. of 1% sodium chloride, no turbidity was produced as observed under the same conditions.

When 1 cc. of 1% sodium chloride was added to a mixture of 25 cc. of water and 5 cc. of 0.1 N silver nitrate, a very marked turbidity was evidenced. These results showed that silver phenobarbital is weakly dissociated.

The solubility of silver phenobarbital was investigated. The addition of 0.02 cc. of 0.1 N silver nitrate to a saturated solution of Phenobarbital U. S. P. XI resulted in the formation of a distinct turbidity which did not disappear after five minutes of stirring. The addition of 0.83 Gm. of anhydrous sodium carbonate caused a disappearance of the turbidity. The addition of sufficient silver nitrate solution again restored the turbidity. It was concluded that silver phenobarbital is insoluble in water but soluble in the presence of sodium carbonate. The appearance of a turbidity on adding silver nitrate the second time was due to reaction between the excess silver nitrate and the sodium carbonate. The removal of silver ions from the field of reaction was shown by the disappearance of turbidity on adding a saturated solution of Phenobarbital U. S. P. XI to a suspension of silver carbonate which had been formed by adding 0.02 cc. of 0.1 N silver nitrate to a solution of 0.83 Gm. of anhydrous sodium carbonate in 25 cc. of water.

To determine whether or not the pH was lowered when phenobarbital was added to a suspension of silver carbonate, the following experiment was conducted: The conditions existing in the Budde assay (93) were duplicated. Determination of pH was made at various stages. The pH of a 3.3% solution of anhydrous sodium carbonate was 11.56 at 25° C. Upon adding 0.02 cc. of 0.1 N silver nitrate, the pH was found to be

11.54 at 25° C. The solution was distinctly turbid. The pH after addition of 1 cc. of a saturated solution of Phenobarbital U. S. P. XI was found to be 11.56 at 25° C. Therefore, suspensions of silver carbonate were cleared by phenobarbital without a lowering of the pH.

As mentioned in the review of the literature, the precipitation of phenylethylacetylurea occurs in deteriorated solutions of phenobarbital sodium. This decomposition product is slightly soluble in water as was shown by evaporating a filtrate obtained from a mixture of phenylethylacetylurea and water which had been shaken for two hours. The reaction of silver nitrate with this compound as it occurs in deteriorated solutions of phenobarbital sodium is a possibility in the Budde assay (38). To determine whether or not the presence of dissolved phenylethylacetylurea in such solutions would introduce an error in the Budde assay, a suspension of this compound was treated with sodium carbonate in the concentration employed in the assay. To 35 cc. of a saturated solution of phenylethylacetylurea containing an excess of the compound, 1.2 Gm. of anhydrous sodium carbonate was added. The mixture was shaken for two hours and then filtered. To 25 cc. of the filtrate, 0.1 N silver nitrate was added. A distinct turbidity was produced by 0.04 cc. This amount of silver nitrate would otherwise indicate the presence of 1 mg. of phenobarbital sodium. The error introduced thereby would be insignificant in the experiments on deterioration of solutions of phenobarbital sodium subsequently discussed.

The possibility of an introduction of error due to the presence of other decomposition products remained to be settled. It was decided to heat a suspension of phenylethylacetylurea under the most

drastic conditions to which solutions in the subsequent experiment on deterioration were subjected, namely, heating at 127° C. for two hours at pH 9.9. In this way the maximum breakdown of the compound into phenylethylacetic acid and urea would occur. To 35 cc. of water, 0.25 Gm. of phenylethylacetylurea was added. It was found to have a pH of 7.7. Sufficient 0.1 N sodium hydroxide was added to raise the pH to 9.9. The mixture was heated in a glass-stoppered pyrex bottle under the conditions just mentioned. The pH fell to 7.6. In the contents of the bottle, 1.2 Gm. of anhydrous sodium carbonate was dissolved. The mixture was filtered and 25 cc. of the filtrate was titrated with 0.1 N silver nitrate. The entire experiment was repeated to provide a check. The average amount of silver nitrate required to produce a distinct turbidity was 0.60 cc. This amount would otherwise indicate the presence of 0.06 Gm. of phenobarbital sodium in 100 cc. It was concluded that the Budde assay was sufficiently accurate to follow the decomposition of solutions of phenobarbital sodium.

Phenobarbital Sodium U. S. P. XI, which had been purchased from the Mallinckrodt Chemical Works with the control designation KLM1, was assayed by the methods of Budde (95), Viebock and Fuchs (95, 96), and Schulek and Rozsa (100, 101). The details of each method were given in the review of the literature. The results which were obtained are outlined in the table which follows.

TABLE 25

COMPARISON OF SEVERAL METHODS OF ASSAY OF PHENOBARBITAL SODIUM U. S. P. XI EMPLOYING SILVER NITRATE

Calculated Dry Sample Gm.	Phenobarbital Gm.	%	Phenobarbital Sodium Gm.	%
		FUDGE ASSAY		
(1) 0.3073	0.276	89.8	0.301	98.1
(2) 0.2598	0.233	89.8	0.255	98.3
(3) 0.2583	0.230	89.2	0.252	97.7
		VIENOUK AND FUCHS ASSAY		
(4) 0.2661	0.235	88.4	0.258	96.8
(5) 0.2637	0.238	90.1	0.260	98.6
(6) 0.2736	0.243	88.8	0.266	97.2
		SCHULEK AND ROZSA ASSAY		
(7) 0.1683	0.146	86.6	0.160	94.8
(8) 0.1798	0.151	84.1	0.166	92.1
(9) 0.2074	0.177	85.4	0.194	93.5

The Budde assays showed the presence of 89.6% phenobarbital in the samples of Phenobarbital Sodium U. S. P. XI. The Viebock and Fuchs assay showed the presence of 89.1% phenobarbital. The Schulek and Rozsa Assay showed the presence of 85.4% phenobarbital. The U. S. P. XII tolerance (71) for phenobarbital is 89.0 to 91.6%. Therefore, the Budde method was considered satisfactory. Although the method of Viebock and Fuchs (95, 96) gave equally accurate results, it was difficult to determine the end point because of the pink color produced upon the addition of silver nitrate in the second part of the assay. The method of Schulek and Rozsa (100, 101) was considered unsatisfactory both from the standpoint of the low results and the difficulty in detecting the end point.

4. Von Babitsch Titration of Phenobarbital

A method was sought for the quantitative determination of the phenobarbital in residue II of the Rotondaro assay (72). The purpose was to eliminate the work involved in drying the residue to constant weight by substituting a volumetric determination of the phenobarbital after the chloroform had been evaporated off. In this experiment the samples employed were residues of phenobarbital obtained in previous Rotondaro assays. The melting points of the individual residues were within the melting point range given in the U. S. P. XII (71). The assay consisted of dissolving 0.1 to 0.2 Gm. of sample in 20 cc. of alcohol, adding 2-3 drops of 0.1% alcoholic solution of thymolphthalein, and titrating with 0.1 N sodium hydroxide to a blue color. The results obtained are given in the table which follows.

TABLE 26

VON BARITSCH TITRATION OF PHENOBARBITAL

Sample Number	m.p.° C.	Weight of Sample Gm.	Amount Found Gm.	Per cent of Sample taken
1	175	0.2328	0.234	100.4
2	175	0.2405	0.241	100.0
3	175	0.2095	0.209	99.8
4	176.8	0.2450	0.242	98.9
5	176.8	0.1621	0.162	99.8
6	176.8	0.1334	0.132	99.0
7	176.1	0.1759	0.177	100.8
8	176.1	0.1845	0.184	99.9
9	176.1	0.2124	0.212	100.0
			Average	99.8

The volumetric determination of pure phenobarbital by the method of von Habitsch (88) gave an average result of 99.6%. It was concluded that the method was sufficiently accurate for the titration of residue II as obtained in the Rotondaro assay (72).

5. Comparison of Assays of a Deteriorated Solution of Phenobarbital Sodium

By assaying a deteriorated solution of phenobarbital sodium with the U. S. P. XI (70), Rotondaro (72), and Budde (93) methods, it was desired to obtain a comparison of the percentage decomposition as indicated by these methods.

Undried Phenobarbital Sodium U. S. P. XI, which had been purchased with the Mallinckrodt control designation XL#1, was used to prepare the solution. The per cent of calculated dry sample in the solution was 4.00 % w/v. Into four-ounce, glass-stoppered, pyrex bottles were introduced 25 cc. portions of the solution. Therefore, 1 Gm. of calculated dry Phenobarbital Sodium U. S. P. XI was in each bottle. The bottles were placed in an autoclave and heated at 115° C. for thirty minutes. Time was taken when the pressure had reached 20 pounds. Eighteen minutes had elapsed since the introduction of the bottles into the chamber.

The U. S. P. XI assay (70) was carried out on each member of a set of three samples by merely doubling the quantities called for in the official procedure, inasmuch as the latter employs 0.5 Gm. of the sample. The phenobarbital was extracted with twelve 25 cc. portions of ether, each period of shaking being not less than ten minutes in

duration.

The modified Rotondaro assay was performed on another set of samples. The method employed was previously described in the section on evaluation of reported methods of assay. The samples were quantitatively transferred to separators with the aid of small portions of water which totaled approximately 35 cc.

In carrying out the Budde assay (23) 3.33 Gm. of anhydrous sodium carbonate was dissolved in the contents of each bottle. The mixture was quantitatively diluted to 100 cc. and filtered through a dry filter. The first 20 cc. of filtrate were discarded. Of the subsequent filtrate, 25 cc. were titrated with a standard solution of silver nitrate. The assay was repeated in each case on another portion of the filtrate.

The results obtained in this experiment are summarized in the following three tables.

TABLE 27

U. S. P. XI ASSAY OF A 4% W/V SOLUTION OF PHENOBARBITAL SODIUM
U. S. P. XI AFTER HEATING AT 115° C. FOR THIRTY MINUTES

Phenobarbital Extracted		Calculated $C_{12}H_{11}O_3N_2Na$		Strength of Solution After Heating	Per Cent Decomposition
Gm.	%	Gm.	%	%	
(1) 0.870	87.0	0.952	95.2	3.81	4.8
(2) 0.881	88.1	0.964	96.5	3.86	3.6
(3) 0.880	88.0	0.963	96.4	3.85	3.7

TABLE 28

MODIFIED ROTONDARO ASSAY OF A 4% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI AFTER HEATING AT 115° C. FOR THIRTY MINUTES

Sample Number	1	2	3
Residue I			
Gm.	0.052	0.024	0.042
per cent	5.2	2.4	4.2
Residue II			
Gm.	0.606	0.595	0.598
per cent	60.5	59.5	59.8
Residue III			
Gm.	0.216	0.173	0.196
per cent	21.6	17.3	19.5
Calculated $C_{12}H_{11}O_3N_2Na$			
Gm.	0.662	0.652	0.643
per cent	66.2	65.2	64.3
Strength of Solution after Heating	2.65%	2.61%	2.57 %
Per cent Decomposition	33.8	34.8	35.8

TABLE 29

BUDDE ASSAY OF A 4% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI AFTER HEATING AT 115° C. FOR THIRTY MINUTES

Sample Number	cc. of 0.1 N $AgNO_3$	$C_{12}H_{11}O_3N_2Na$		Strength of Solution After Heating	Per Cent Decomposition
		Gm.	%	%	
1	8.20	0.209	83.5	3.34	16.5
	8.19	0.208	83.4	3.33	16.6
2	8.20	0.209	83.5	3.34	16.5
	8.20	0.209	83.5	3.34	16.5
3	8.18	0.208	83.3	3.33	16.7
	8.18	0.208	83.3	3.33	16.7

The U. S. P. XI assays showed 3.7% decomposition in the 4% w/v solution of Phenobarbital Sodium U. S. P. XI which had been heated at 115° C. for thirty minutes. The modified Rotondaro assay showed 34.7% decomposition and the Budde assay showed 16.6% decomposition. Therefore, checks were not obtained for the determination of deterioration by the several methods mentioned.

The U. S. P. XI method gave results for percentage deterioration which are obviously lower than the true decomposition, since in this method most of the products of deterioration are evaluated as undecomposed phenobarbital. The results for percentage deterioration by the modified Rotondaro method are considered to be too high, since previous results indicated that the modified Rotondaro method, as carried out in the present investigation, failed to recover all the unchanged phenobarbital. The Budde method, which gave results for percentage

deterioration between those obtained by the U. S. P. XI method and the modified Rotondaro method, was selected for further use in determining percentage deterioration in the present investigation. From the studies made of the Budde method, it appears that it gives results that are approximately correct or that are sufficiently accurate for determining the relative degree of deterioration in comparative tests.

E. A STUDY OF A NUMBER OF FACTORS IN THE DETERIORATION OF SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI

It was desired to investigate the effects produced by variations in time, temperature, and concentration on such factors in the deterioration of solutions of phenobarbital sodium as percentage deterioration, pH, volume of precipitate, and refractive index. This work was prompted by the results reported by Nielsen (13), Madsen (3), Aspelund and Skoglund (7), and Tomski and Waller (24). Madsen (3) reported results regarding pH change and percentage decomposition obtained on heating a 10% solution of diethylbarbituric acid at 60°, 80°, and 100° C. Nielsen (13) showed the relation of pH to decomposition of a 10% solution of phenobarbital sodium at temperatures not exceeding 39° C. Aspelund and Skoglund (7) reported the quantitative isolation of various decomposition products of phenobarbital sodium in solutions which had been boiled for three hours, and in solutions which had been stored for eight days. Tomski and Waller (24) investigated the percentage deterioration of solutions of phenobarbital sodium at various temperatures for various periods of time. In an attempt to verify and extend the data presented by these workers, experiments were designed

to include, in whole or in part, an investigation of factors previously studied and at the same time to amplify, and to fill in the hiatuses, and to study additional factors in the deterioration of solutions of one barbiturate, namely, phenobarbital sodium. The temperature and periods of exposure were selected to encompass most of the conditions previously employed by the aforeto mentioned workers and to include for the most part the conditions of sterilization recommended for solutions intended for parenteral use as given in the U. S. P. VI (73), the British Pharmacopoeia, 1932 (26), and the United States Dispensatory (106). In this manner the changes occurring under conditions of sterilization and conditions reported by other workers were investigated, providing at the same time an insight into deterioration taking place during prolonged storage through the agency of these accelerated tests.

In this series of experiments, 5, 10, and 20% solutions of Phenobarbital Sodium U. S. P. XI were employed. In every case, 25-cc. portions of the solution were heated in 125-cc., glass-stoppered, pyrex bottles. Unless otherwise indicated each of the titrations given in the tables represents the results obtained from the titration of the contents of a separate bottle. The group of determinations which includes volume of precipitate, refractive index, and pH were made on the contents of a separate bottle. In the event a precipitate had been produced, the clear supernatant liquid which remained after centrifuging was employed in making the determination of refractive index. The instrument used in making this determination was the Abbe Refractometer, the temperature of whose prisms were thermostatically controlled by the flow of water, maintained at 25 ($\pm$0.5)° C., through the surrounding

jackets. The use of the autoclave, the determination of pH, and volume of precipitate were previously described.

From the investigation conducted on the evaluation of methods of assay, it was considered advisable to employ the Budde method in following the deterioration. The original Budde method (93) was reported as follows: About 0.2 to 0.3 Gm. of the sodium salt of the barbiturate is weighed and dissolved in 50 cc. of water, together with 1 Gm. of anhydrous sodium carbonate. To the clear solution 0.1 N silver nitrate is added until a persistent, distinct turbidity is produced. One molecular weight of silver nitrate is equivalent to one molecular weight of the barbiturate.

Inasmuch as solutions of various strengths were employed, appropriate volumes had to be taken and appropriate dilutions had to be made in order to duplicate the conditions of the Budde assay. The procedures followed in all of the assays of this series of experiments are outlined in the table which follows.

TABLE 30

PROCEDURE FOLLOWED IN THE BUDDE ASSAY OF 5, 10, and 20% SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI

Percentage Strength of the solution studied		5	10	20
Volume of each solution placed in each bottle	(cc.)	25	25	25
Dilution made for each 25 cc. of solution	(cc.)	125	250	500
Amount of anhydrous Na_2CO_3 added before dilution	(Gm.)	4.17	8.33	16.67
Alternative amount of anhydrous Na_2CO_3 to add to each 25 cc. aliquot	(Gm.)	0.83	0.83	0.83
25 cc. of the dilution was taken for the titration. This volume represented (x) cc. of the original solution		5	2.5	1.25

In all of the experiments of this series, undried samples were used to prepare the solutions. The per cent of moisture in the drug was determined on separate samples before preparation of the solutions. The strength of each solution was based on the calculated amount of dry sample dissolved. The solutions were assayed before and after treatment. If a precipitate was present, the contents of the bottle, in which the solution was kept during the treatment, were quantitatively transferred to a volumetric flask. Before dilution the prescribed amount of anhydrous

sodium carbonate was dissolved in the mixture. The sodium carbonate was dissolved before dilution in order to redissolve any possible phenobarbital which may have been precipitated. This amount of anhydrous sodium carbonate will dissolve the maximum amount of phenobarbital that can be formed from the amount of phenobarbital sodium which was present in the aliquot part of any solution studied. In a previous experiment using 1 Gm. of Phenobarbital U. S. P. XI, 2.8 Gm. anhydrous sodium carbonate, and 25 cc. of water, it was found that a clear solution was produced after stirring for four minutes. Eight minutes of agitation of the sodium carbonate with the contents of the bottle was considered ample time in which to dissolve any free phenobarbital which may have been present. After dilution a portion of the mixture was filtered through a dry filter. The first 20 cc. of the filtrate was discarded. Of the subsequent filtrate 25 cc. was taken for the titration. In the event no precipitate had formed in the solution during the heating, sodium carbonate was not added until the filtration had been performed, at which time a proportional part of the sodium carbonate as given in Table 30 was added to the aliquot part of the solution which was to be titrated.

It was deemed advisable to determine whether or not there would be any significant difference between the results obtained by the Budde method if the deteriorated solution in the bottle was quantitatively passed through a filter before dilution instead of diluting the deteriorated solution containing the precipitate to the same volume. Towards this end, four 25 cc. portions of a 10% solution of Phenobarbital Sodium U. S. P. XI (Mallinckrodt Control XXM) were heated at 115° C.

for one hour during which time approximately 16 cc. of precipitate per 100 cc. of solution was formed. The actual volume of the precipitate was probably considerably less because the latter mentioned determination of volume of precipitate did not take into consideration the occluded solution nor the free phenobarbital which may have been present and which would have been dissolved by the sodium carbonate. Two of the samples were diluted to 250 cc. before filtration and two were filtered before dilution. In each case the sodium carbonate was first of all dissolved in the contents of the bottle. Portions of the solution were assayed before the treatment. The results are given in the table which follows.

TABLE 31

EFFECT ON THE SOLUBILITY ASSAY ON REMOVING THE PRECIPITATE FROM A DETERIORATED SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI IN TWO DIFFERENT WAYS

	Precipitate filtered out before dilution		Precipitate filtered out after dilution	
Period of Heating Minutes	0	60	0	60
cc. of 0.1 N $AgNO_3$ for 2.5 cc. aliquot	9.59	7.57 7.56	9.59	7.52 7.53
Per cent $C_{12}H_{11}O_3N_2Na$ in solution	9.75	7.69	9.75	7.65
Apparent deterioration, per cent		21.1		21.5

No appreciable difference in results concerning percentage deterioration of a 10% solution of Phenobarbital Sodium U. S. P. XI which had been heated at 115° C. for thirty minutes was found on employing the Budds method in two different ways, namely, (a) removal of the precipitate formed in deteriorated solution before dilution, and (b) removal of the precipitate after dilution. The difference between the results on percentage decomposition was 0.4%.

Therefore, it was concluded that the determination of percentage deterioration could be made by transferring the deteriorated solution together with the precipitate to a volumetric flask with subsequent filtration after dilution without incurring appreciable error.

1. Effect of Heating a 5% w/v Solution of Phenobarbital Sodium U. S. P. XI at 80° C.

The deterioration and change in pH of a 5% w/v solution of Phenobarbital Sodium U. S. P. XI upon exposure to a temperature of 80 (±0.5)° C. for periods of time ranging from 15 to 90 minutes were determined.

The lot of drug employed was purchased with the Mallinckrodt control designation KLML. The solution was heated in a thermostat. The thermostat mentioned in this and the subsequent experiment, which was carried out at 80° C., was regulated by a De Khotinsky Thermoregulator. The temperature was observed by means of a mercury thermometer calibrated in tenths of a degree. The water in the thermostat was kept in constant motion by means of an electric stirrer.

On separate occasions, four solutions of the same strength were prepared from the same lot of drug. Before heating each solution was

assayed three times and the pH of each was determined at least twice. In 125-cc., glass-stoppered, pyrex bottles were placed 25-cc. portions of the solution. Each result given in Table 32 concerning the per cent phenobarbital sodium remaining in a treated solution represents the average of six assays conducted twice on the contents of each of three bottles. Time was taken when the bottles were immersed in the thermostat. They were withdrawn at the end of the time interval and immediately inserted into a mixture of ice and water to cool the solution rapidly. The determination of pH was made on separate 25-cc. portions of the solution which had been treated in the manner just described.

From the analysis of the untreated solutions it was found that the per cent of phenobarbital sodium in the lot employed was 97.4%. In the previous experiment on comparison of assays employing silver nitrate, the Budde assay gave 98.0% phenobarbital sodium.

The results obtained in this experiment are summarized in the table which follows.

TABLE 32.

EFFECT OF HEATING A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI AT 60 (±0.5)° C.

Time Minutes	Solution Number	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	pH at	°C.
0	1	4.9		9.53	29
0	2	4.9		9.51	28
0	3	4.9		9.49	28
0	4	4.9		9.48	30
	Averages	4.9	0.0	9.51	29
15	1	4.8		9.51	28
15	2	4.9		9.51	28
	Averages	4.9	0.0	9.51	28
30	2	4.9	0.0	9.49	27
60	3	4.8	2.0	9.43	29
90	4	4.8	2.0	9.50	30

From Table 32, it is seen that 5% solutions of Phenobarbital Sodium U. S. P. XI underwent no deterioration when heated at 80° C. for 15 or 30 minutes. Percentage deterioration amounted to 2.0% after exposure for 60 and 90 minutes. The pH of the solution remained the same at the end of 15 and 30 minutes. The determination of pH at the end of 60 and 90 minutes was in doubt because of the lack of regularity in results obtained. No precipitate was evident in any of the exposed solutions.

The N. F. VI directs that fractional sterilization at low temperature be carried out by heating the solution at 60° to 70° C. for 30 minutes on three successive days. Therefore, it was concluded that 2.0% deterioration might occur in this particular solution when treated at 80° C. under the conditions referred to in the N. F. VI (73).

2. Effect of Heating 5, 10, and 20% w/v Solutions of Phenobarbital Sodium U. S. P. XI at 80° C.

The purpose of the experiment was to determine the effects produced by heating 5, 10, and 20% solutions of Phenobarbital Sodium U. S. P. XI at 80° C. for 15, 30, and 60 minutes. As an emergency method of sterilization, the British Pharmacopoeia, 1932 (28) directs heating the solution at 80° C. for not less than 30 minutes.

In the previous experiment on the deterioration of a 5% solution at 80° C., the sample employed was that purchased from the Mallinckrodt Chemical Works bearing the control designation KLM1. In this experiment the sample used was purchased from the same company but it had the control designation KKM. In the event that there might be some difference

between the samples, a 5% solution of the former sample was prepared. The results of its assay and determination of deterioration after heating at 80° C. for 15 minutes were compared with corresponding results obtained from a 5% solution of the latter sample.

Table 33 indicates the results obtained with a 5% solution prepared from the sample which was purchased with the Mallinckrodt control designation KLM1.

TABLE 33

EFFECT OF HEATING A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 80 (±0.3)° C.

Time Minutes	cc. 0.1 N $AgNO_3$ for 5 cc. aliquot	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	pH at	°C.
0	9.63			9.38	31
0	9.61			9.38	31
Averages	9.62	4.89		9.37	31
15	9.58			9.31	32
15	9.64			9.31	32
Averages	9.66	4.86	0.6	9.31	32

*The Phenobarbital Sodium U. S. P. XI was purchased with the Mallinckrodt control designation KLM1. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.8%. No precipitate was evident in any of the solutions.

TABLE 34

EFFECT OF HEATING A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 80 (±0.5)° C.

Time Minutes	cc. 0.1 N $AgNO_3$ for 5 cc. aliquot	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	pH at °C.	
0	9.58			9.52	30
0.	9.58			9.54	30
Averages	9.58	4.87		9.53	30
15	9.53			9.59	29
15	9.54			9.60	29
15	9.54				
Averages	9.54	4.85	0.4	9.60	29
30	9.48			9.48	29
30	9.51			9.48	29
30	9.49				
Averages	9.49	4.82	1.0	9.48	29
60	9.43			9.38	29
60	9.41			9.38	29
60	9.42				
Averages	9.42	4.79	1.6	9.37	29

*The Phenobarbital Sodium U. S. P. XI was purchased with the Mallinckrodt control designation KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.4%. No precipitate was evident in any of the solutions except those which had been heated for 60 minutes. The latter possessed a very slight amount of precipitate.

TABLE 35

EFFECT OF HEATING A 10% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 80 (±0.6)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	Precipitate cc./100 cc.	pH at °C.	
0	9.70		0	9.63	30
0	9.68		0	9.62	30
Averages	9.69		0	9.63	30
15	9.68		0	9.60	30
15	9.69		0	9.60	30
15	9.69		0		
Averages	9.69	0.0	0	9.60	30
30	9.66		0	9.56	29
30	9.66		0	9.56	29
30	9.64		0		
Averages	9.65	0.4	0	9.56	29
60	9.51		1	9.40	29
60	9.52		1	9.42	29
60	9.51				
Averages	9.51	1.9	1	9.41	29

*The Phenobarbital Sodium U. S. P. XI was purchased with the Mallinckrodt control designation KEM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 98.9%.

TABLE 36

EFFECT OF HEATING A 20% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 80 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	Precipitate cc./100 cc.	pH at °C.	
0	19.62		0	9.70	31
0	19.62		0	9.72	31
Averages	19.62		0	9.71	31
15	19.45		0	9.69	30
15	19.50		0	9.69	30
15	19.45				
Averages	19.47	0.8	0	9.69	30
30	19.35		0	9.64	30
30	19.31		0	9.68	30
30	19.31				
Averages	19.33	1.5	0	9.65	30
60	19.03		2	9.50	30
60	19.02		3	9.52	30
Averages	19.03	3.0	3	9.51	30

*The Phenobarbital Sodium U. S. P. XI was purchased with the Mallinckrodt control designation KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 98.1%.

TABLE 37

PER CENT DETERIORATION AND pH CHANGE OF 5, 10, AND 20% SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI HEATED AT 80 (±1)°C.

Strength of Solution	5%	10%	20%
Unheated Solution			
pH	9.53	9.63	9.71
15 minutes			
Per cent deterioration	0.4	0.0	0.8
pH	9.60	9.60	9.69
30 minutes			
Per cent deterioration	1.0	0.4	1.6
pH	9.48	9.56	9.65
60 minutes			
Per cent deterioration	1.6	1.9	3.0
pH	9.37	9.41	9.51

The results obtained by means of the Budds assay of two 5% solutions of Phenobarbital Sodium U. S. P. XI, prepared from two different lots of drug, both of which solutions were heated at 80° C. for 15 minutes, showed 0.6% deterioration in the solution prepared from the lot designate KLM1 and 0.4% deterioration in the solution prepared from the lot designated KK%. It was concluded that these results indicated no significant difference between the two lots of drug which would prevent comparison of results obtained in this study of deterioration.

In Table 37 the results obtained on heating 5, 10, and 20% solutions at 80° C. for 15, 30, and 60 minutes are summarized. An increasing drop in pH was found to have occurred on heating each solution at successively longer periods of time. An exception to this is to be noted in the case of the 5% solution heated for 15 minutes. It was felt that this represented an error. Likewise an increase in percentage deterioration was noted for each separate solution. Table 37 indicates that 20% solutions showed somewhat greater deterioration than 5 and 10 % solutions. The results of percentage deterioration of the 20% solution were comparable to those obtained by Tomaki and Waller (24). These workers found 1.0 to 1.6% deterioration at the end of 30 minutes and 2.0 to 3.0% deterioration at the end of 60 minutes. In the present study, 1.5% and 3.0% deterioration, respectively, were found. As an emergency method of sterilisation, the British Pharmacopoeia, 1932 (26) directs heating solutions at 80° C. for 30 minutes. Under these conditions, 5% and 20% solutions underwent 1.0% and 1.5% deterioration, respectively.

3. Effect of Heating 5, 10, and 20% w/v Solutions of Phenobarbital Sodium U. S. P. XI at 100° C.

The purpose of the experiment was to determine the effects of heating 5, 10, and 20% w/v solutions of Phenobarbital Sodium U. S. P. XI at 100° C. for 15, 30, and 60 minutes.

The bottles containing the solutions were exposed to steam for the periods of time mentioned. The bottles were set in a tray above boiling water contained in an autoclave. The lid was immediately replaced after the introduction of the tray. As soon as the temperature of the surroundings above the water had reached 100° C., as indicated by a mercury thermometer, the time was taken by means of a stopwatch. The temperature was rapidly brought to 100° C. by heating the autoclave with the aid of several Meker burners. The petcock was allowed to remain open during the entire heating period to allow escape of steam. At the end of the period the bottles were immediately removed and cooled by immersion into a mixture of ice and water.

TABLE 38

EFFECT OF HEATING A 6% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 100 (±0.5)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at °C.		Refractive Index at 25° C.
0	4.87		0	9.53	31	1.3432
0	4.87		0	9.53	31	1.3431
Averages	4.87		0	9.53	31	1.3432
15	4.77		0	9.42	30	1.3432
15	4.77		0	9.43	29	1.3432
15	4.78					
Averages	4.77	2.1	0	9.43	30	1.3432
30	4.67		2	9.23	28	1.3429
30	4.66		2	9.22	28	1.3429
30	4.67					
Averages	4.67	4.1	2	9.23	28	1.3429
60	4.39		4**	9.01	29	1.3428
60	4.39		4**	9.01	28	1.3428
60	4.37					
Averages	4.38	10.1	4	9.01	29	1.3428

*The substance used to prepare the solution bore the Mallinckrodt control designation KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.4%.

**Ten (10) cc. of solution were heated in a corked centrifuge tube.

TABLE 39

EFFECT OF HEATING A 10% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 100 (±0.5)° C.

Time in Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	Per cent Deterioration	Precipitate cc./100 cc.	pH at °C.	
0	9.77		0	9.63	31
0	9.77		0	9.61	31
Averages	9.77		0	9.62	31
15	9.58		0	9.40	31
15	9.58		0	9.41	31
15	9.58				
Averages	9.57	2.1	0	9.41	31
30	9.28		4	9.23	31
30	9.28		4	9.23	31
30	9.28				
Averages	9.28	5.1	4	9.23	31
60	8.81		8	9.04	29
60	8.80		8	9.06	29
60	8.78				
Averages	8.80	9.9	8	9.05	29

*The substance used to prepare the solution bore the Mallinckrodt control number NKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.7%.

TABLE 40

EFFECT OF AERATING A 20% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 100 (±0.5)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at	°C.	Refractive Index at 25° C.
0	19.58		0	9.80	28	1.3750
0	19.54		0	9.80	28	1.3750
Averages	19.56		0	9.80	28	1.3750
15	19.13		1	9.80	28	1.3750
15	19.15		1	9.82	28	1.3750
15	19.17					
Averages	19.15	2.1	1	9.81	28	1.3750
30	18.52		4	9.37	28	1.3740
30	18.52		5	9.38	28	1.3740
30	18.52					
Averages	18.52	5.3	5	9.38	28	1.3740
60	17.67		14	9.14	28	1.3722
60	17.67		13	9.15	28	1.3721
60	17.69					
Averages	17.67	9.6	14	9.15	28	1.3722

*The substance used to prepare the solution bore the Mallinckrodt control designation KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.8%.

TABLE 41

EFFECT OF HEATING 5, 10, and 20% W/V SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI AT 100 (±0.5)° C.

Strength of Solution	5%	10%	20%
Unheated Solution			
pH	9.53	9.62	9.80
Precipitate cc./100 cc.	0	0	0
15 minutes			
Per cent deterioration	2.1	2.1	2.1
pH	9.43	9.41	9.61
Precipitate cc./100 cc.	0	0	1
30 minutes			
Per cent deterioration	4.1	5.1	5.3
pH	9.23	9.28	9.38
Precipitate cc./100 cc.	2	4	5
60 minutes			
Per cent deterioration	10.1	9.9	9.6
pH	9.01	9.05	9.16
Precipitate cc./100 cc.	4	8	14

In Table 41, the results obtained on heating 5, 10, and 20% solutions at 100° C. for 15, 30, and 60 minutes are summarized. A continuous drop in pH was found to have occurred on heating each solution for successively longer periods of time. The percentage deterioration at a single period of heating remained approximately the same regardless of the strength of the original solution. An increase in the amount of precipitate was noted with increase in concentration of the original solution. A comparatively large decrease in refractive index of the solution appeared to take place upon the formation of a large amount of precipitate. Tomski and Waller (24) stated that 1.0%

deterioration took place in a 20% solution heated at 100° C. for 15 minutes and 9.0 to 10.5% deterioration at the end of 60 minutes of heating. In the present study, 2.1% and 9.8% deterioration, respectively were found under these conditions.

4. Effect of Heating 5, 10, and 20% w/v Solutions of Phenobarbital Sodium U. S. P. XI at 115° C.

The purpose of the experiment was to determine the effects of heating 5, 10, and 20% w/v solutions of Phenobarbital Sodium U. S. P. XI at 115 (±1)° C. for 15, 30, 60, and 120 minutes. Inasmuch as the N. F. VII (78) prescribes sterilization of ampuls at 115.5° C., it was deemed appropriate to select this temperature at which to heat the solutions. The results obtained are given in Tables 42, 43, and 44.

TABLE 42

EFFECT OF HEATING A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 115 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at	°C.	Refractive Index at 25° C.
0	4.90		0	9.48	27	1.3432
0	4.88		0	9.47	27	1.3432
Averages	4.89		0	9.48	27	1.3432
15	4.70		2	9.21	27	1.3431
15	4.70		2	9.23	27	1.3430
Averages	4.70	3.9	2	9.22	27	1.3431
30	4.28		4	8.92	27	1.3427
30	4.27		4	8.91	27	1.3428
Averages	4.27	12.7	4	8.92	27	1.3428
60	3.81		5	8.68	28	1.3422
60	3.82		5	8.68	28	1.3422
Averages	3.82	21.9	5	8.68	28	1.3422
120	3.09		9	8.51	28	1.3412
120	3.06		10	8.52	28	1.3411
Averages	3.08	37.0	10	8.52	28	1.3412

*The substance used to prepare the solution bore the Mallinckrodt control number MKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare this solution was 97.8%.

TABLE 43

EFFECT OF HEATING A 10% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 115 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at	°C.	Refractive Index at 25° C.
0	9.79		0	9.62	28	1.3540
0	9.80		0	9.62	28	1.3540
Averages	9.80		0	9.62	28	1.3540
15	9.31		4	9.28	28	1.3537
15	9.28		5	9.28	28	1.3537
Averages	9.29	5.2	4	9.28	28	1.3537
30	8.71		9	8.91	28	1.3523
30	8.71		10	8.92	28	1.3523
Averages	8.71	11.1	10	8.92	28	1.3523
60	7.62		15	8.99**	29	1.3505
60	7.59		14	8.98**	29	1.3504
Averages	7.61	22.3	15	8.99**	29	1.3505
120	6.38		18	8.93**	28	1.3488
120	6.36		14	8.93**	28	1.3487
Averages	6.37	35.0	16	8.93	28	1.3488

*The substance used to prepare the solution bore the Mallinckrodt control number KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 98.0%.

**The presence of free ammonia was detected by odor and by moistened litmus paper suspended over the solution. The presence of carbon dioxide was detected by Eltesta reagent paper (22).

TABLE 44

EFFECT OF HEATING A 20% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 115 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at °C.		Refractive Index at 25° C.
0	19.74		0	9.76	30	1.3756
0	19.73		0	9.76	30	1.3756
Averages	19.74		0	9.76	30	1.3756
15	18.52		4	9.35	30	1.3748
15	18.52		4	9.34	30	1.3747
Averages	18.52	6.2	4	9.35	30	1.3748
30	17.61		9	9.13	29	1.3737
30	17.52		13	9.10	29	1.3732
Averages	17.57	11.0	11	9.12	29	1.3735
60	15.53		33	8.35**	29	1.3687
60	15.55		31	8.36**	29	1.3689
Averages	15.54	21.2	32	8.35**	29	1.3688
120	12.69		48	8.60**	29	1.3631
120	12.71			8.62**	29	
Averages	12.70	35.6	48	8.61**	29	1.3631

*The substance used to prepare the solution bore the Mallinckrodt control number KKM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 98.6%.

**The pH was determined immediately after opening the bottle. The pH rose from the figure given in the table to a figure from pH 0.3 to pH 0.5 higher. The maximum pH was not ascertained. The presence of ammonia was detected by suspending moistened litmus paper over the solution.

TABLE 45

EFFECT OF HEATING 5, 10, and 20% W/V SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI AT 115 (±1)° C.

Strength of Solution		5%	10%	20%
Unheated Solution				
	pH	9.48	9.62	9.76
Precipitate	cc./100 cc.	0	0	0
15 minutes				
Per cent deterioration		5.9	5.2	6.2
	pH	9.22	9.28	9.35
Precipitate	cc./100 cc.	2	4	4
30 minutes				
Per cent deterioration		12.7	11.1	11.0
	pH	8.92	8.92	9.12
Precipitate	cc./100 cc.	4	10	11
60 minutes				
Per cent deterioration		21.9	22.3	21.2
	pH	8.68	8.98*	8.85
Precipitate	cc./100 cc.	6	15	32
120 minutes				
Per cent deterioration		37.0	35.0	35.6
	pH	8.52	8.93*	8.61
Precipitate	cc./100 cc.	10	16	48

*The failure of the pH to continue to fall below that found at the end of thirty minutes of heating was probably due to the presence of ammonia which was detected upon opening the bottles containing the solution.

In Table 45 the results obtained on heating 5, 10, and 20% solutions at 115 (±1)° C. for 15, 30, 60, and 120 minutes are summarized. A continuous drop in pH was found on heating 5% and 20% solutions at successively longer periods of time. In the case of the 10% solution the pH failed to fall below that found at the end of 30 minutes of heating. This was probably due to formation of ammonia. No marked difference in percentage deterioration at a single period of heating was found to occur in solutions of different strengths. An increase in amount of precipitate was generally indicated with increase in concentration of the original solutions. Tomski and Waller (24) stated that 18.0 to 20.5% deterioration took place in approximately 20% solutions heated at 115° C. for 30 minutes. In the present investigation, 11.0% deterioration at the end of 30 minutes, and 21.2% deterioration at the end of 60 minutes was detected on heating 20% solutions at 116° C. The N. F. VII (78) prescribes the sterilization of ampuls at 115.5° C. for 30 minutes.

5. Effect of Heating 5, 10, and 20% w/v Solutions of Phenobarbital Sodium U. S. P. XI at 127° C.

The purpose of the experiment was to determine the effects of heating 5, 10, and 20% w/v solutions of Phenobarbital Sodium U. S. P. XI at 127 (±1)° C. These solutions were heated at this temperature because it is one of the temperatures prescribed by the N. F. VI (73) and N. F. VII (78) for sterilization of ampuls. The results obtained are given in Tables 46, 47, and 48.

TABLE 48

EFFECT OF HEATING A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 127 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at	°C.	Refractive Index at 25° C.
0	4.88		0	9.59	29	1.3433
0	4.88		0	9.60	29	1.3433
Averages	4.88		0	9.60	29	1.3433
15	4.20		5	8.80	29	1.3424
15	4.20		5	8.82	29	1.3425
Averages	4.20	13.9	5	8.81	29	1.3425
30	3.73		9	8.67**	28	1.3418
30	3.72		8	8.63**	28	1.3419
Averages	3.73	23.6	9	8.65	28	1.3419
60	3.09		10	8.43**	28	1.3411
60	3.05		10	8.44**	28	1.3411
Averages	3.07	37.1	10	8.44	28	1.3411
120	2.12		17	8.16**	29	1.3400
120	2.11					
Averages	2.12	56.6	17	8.16	29	1.3400

*The substance used to prepare the solution bore the Mallinckrodt control number KKN. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.6%.

**The presence of ammonia was detected by odor and by suspending moistened litmus paper over the solution. The pH was determined immediately after opening the bottle.

TABLE 47

EFFECT OF HEATING A 10% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 127 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Fpt. cc. per 100 cc.	pH at	°C.	Refractive Index at 25° C.
0	9.75		0	9.73	25	1.3541
0	9.76		0	9.72	25	1.3540
Averages	9.76		0	9.73	25	1.3541
15	8.83		11	9.25	24	1.3524
15	8.88		8	9.23	24	1.3533
Averages	8.86	11.3	10	9.24	24	1.3529
30	7.83		17	8.73**	23	1.3500
30	7.86		18		24	1.3501
Averages	7.85	22.6	18	8.73	24	1.3501
60	6.21		21	8.52**	24	1.3480
60	6.18		26	8.60**	24	1.3480
Averages	6.20	36.5	24	8.61	24	1.3480
120	4.83		26	8.62**	24	
120	4.82		26	8.64**	24	
Averages	4.83	53.6	27	8.63	24	

*The substance used to prepare the solution bore the Mallinckrodt control number XRM. The amount of phenobarbital sodium in the calculated dry sample used to prepare the solution was 97.6%.

**The pH was determined immediately after opening the bottle. The pH rose from the figure given in the table to a figure from pH 0.1 to pH 0.7 higher. The maximum pH was not ascertained. The presence of ammonia was detected by suspending moistened litmus paper over the solution.

TABLE 48

EFFECT OF HEATING A 20% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI* AT 127 (±1)° C.

Time Minutes	Per cent $C_{12}H_{11}O_3N_2Na$ in solution	% Deterioration	Ppt. cc. per 100 cc.	pH at °C.		Refractive Index at 25° C.
0	19.54		0	9.90	25	1.3757
0	19.56		0	9.92	25	1.3756
Averages	19.55		0	9.91	25	1.3757
15	17.26		13	9.48	25	1.3720
15	17.24		12	9.49	25	1.3720
Averages	17.25	11.8	13	9.49	25	1.3720
30	15.21		18	9.28	25	1.3677
30	15.19		16	9.30	25	1.3680
Averages	15.20	22.3	17	9.29	25	1.3679
60	12.28		30	9.08	25	1.3617
60	12.30		40	9.11	25	1.3618
Averages	12.29	37.1	35	9.10	25	1.3618
120	9.01		**	8.37	25	1.3582
120	8.97		**	8.34	25	1.3568
Averages	8.99	54.0		8.36	25	1.3575

*The substance used to prepare the solution bore the Mallinckrodt control number MKM. The amount of phenobarbital sodium in the calculated dry sample used to make the solution was 97.8%.

**The precipitate had agglomerated into large, hard masses.

TABLE 49

EFFECT OF HEATING 5, 10, and 20% W/V SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI AT 127 (±1)° C.

Strength of Solution	5%	10%	20%
Unheated Solution			
pH	9.80	9.73	9.91
Precipitate cc./100 cc.	0	0	0
15 minutes			
Per cent deterioration	13.9	11.5	11.8
pH	8.81	9.24	9.49
Precipitate cc./100 cc.	6	10	15
30 minutes			
Per cent deterioration	23.6	22.6	22.3
pH	8.65*	8.73**	9.29*
Precipitate cc./100 cc.	9	18	17
60 minutes			
Per cent deterioration	37.1	36.5	37.1
pH	8.44*	8.81**	9.10*
Precipitate cc./100 cc.	10	24	35
120 minutes			
Per cent deterioration	56.6	53.6	54.0
pH	8.16*	8.63**	8.36*
Precipitate cc./100 cc.	17	24	***

*The presence of ammonia was detected. The pH was determined immediately after opening the bottle.

**The presence of ammonia was detected. The pH was determined immediately after opening the bottle. The pH rose from the figure indicated to a figure from pH 0.1 to pH 0.7 higher. The maximum pH was not ascertained.

***The precipitate had agglomerated into large, hard masses.

In Table 48 the results obtained on heating 5, 10, and 20% solutions at 127 (±1)° C. for 15, 30, 60, and 120 minutes are summarized. A continuous drop in pH was found on heating 5 and 20% solutions at successively longer periods of time. In the case of the 10% solution the pH failed to fall below that found at the end of 60 minutes of heating. This was probably due to the formation of ammonia. Ammonia was detected in all solutions after heating for 30, 60, and 120 minute periods. No marked difference in percentage deterioration at a single period of heating was found to occur in solutions of different strengths. An increase in amount of precipitate was indicated with increase in concentration of the original solutions. As a method of sterilization of filled ampuls, the N. F. VII (78) prescribes heating at 126.5° C. for 15 minutes. Under these conditions, 14%, 11%, and 12% deterioration resulted in 5, 10, and 20% solutions, respectively.

F. RELATION OF pH TO THE DETERIORATION OF SOLUTIONS OF PHENOBARBITAL SODIUM

Inasmuch as several workers (2, 8, 10, 16) indicated that the deterioration of solutions of barbiturates decreased upon lowering the pH, an attempt was made to ascertain whether or not there was a particular pH at which solutions of phenobarbital sodium could be stabilized. Moreover it was desirable to learn the pH at which a solution of phenobarbital sodium would precipitate phenobarbital.

1. The Use of Monosodium and Disodium Phosphates.

A buffer mixture was sought to lower the pH of a 5% solution of Phenobarbital Sodium U. S. P. XI. Monosodium and disodium phosphates were selected as components of such a buffer mixture because these compounds are official in the U. S. P. XI (70) and because phosphates are present in the blood stream. Anhydrous dibasic sodium phosphate A. R. and monobasic sodium phosphate A. R. ($NaH_2PO_4 \cdot 1\ H_2O$) were purchased from the Mallinckrodt Chemical Works.

In conducting the experiment, a 15% w/v solution of Phenobarbital Sodium U. S. P. XI was prepared. To 20-cc. portions of this solution various amounts of each of the components of the buffer mixture were added so that the final concentration of the phenobarbital sodium was 5% w/v. The pH of the resulting solution was determined. By substituting water in each instance for the solution of phenobarbital sodium, a comparison between the pH of the buffer mixture and the pH of the buffered solutions of the barbiturate was afforded. When the formation of a precipitate was noted a determination of the volume of precipitate was

undertaken. For this purpose the buffered solutions of the barbiturate were prepared in 50-cc., graduated centrifuge tubes. The remainder of the procedure was followed as previously described for the determination of volume of precipitate. Maintaining the same ratio of buffer and phenobarbital sodium, 25-cc. samples were prepared in four-ounce, glass-stoppered pyrex bottles. The samples were heated at 115 ($\pm$ 1)° C. for thirty minutes. It was found that a 5% solution of Phenobarbital Sodium U. S. P. XI produced approximately 10 cc. of precipitate per 100 cc. Therefore, if a marked decrease in amount of precipitate occurred, a stabilizing effect might be indicated. In addition, these conditions of treatment were chosen because of their known applicability to sterilization. According to Russ (109) moist heat at 115° C. kills both bacteria and spores in less than 30 minutes.

The pH of various proportions of the components of the buffer mixture is given in Table 50. The results obtained in the use of these same buffer mixtures with a 5% w/v solution of Phenobarbital Sodium U. S. P. XI are given in Table 51.

In Table 51 it is seen that the phosphate buffers lowered the pH of a 5% w/v solution of Phenobarbital Sodium U. S. P. XI from pH 9.7 through various pH levels to pH 8.7. Precipitation of phenobarbital from a 5% solution was shown to occur at pH 8.8. The fact that the amount of precipitate was greater in some instances without any distinct difference in pH was probably accounted for by the increase in buffer capacity in those solutions which had larger amounts of sodium biphosphate. The pH of each of the buffered solutions was lower after heating.

Samples which had approximately 12 to 14 cc. of precipitate per 100

TABLE 50

pH OF MIXTURES OF MONOSODIUM AND DISODIUM PHOSPHATE SOLUTIONS

cc. 0.1 M NaH_2PO_4	cc. 0.1 M Na_2HPO_4	cc. Water	pH at	° C.
20	0	10	4.47	26
18	2	10	5.81	26
16	4	10	6.15	26
14	6	10	6.40	26
12	8	10	6.60	25
10	10	10	6.74	26
8	12	10	6.93	26
6	14	10	7.11	25
4	16	10	7.35	26
2	18	10	7.69	25
1	19	10	8.01	25
0	20	10	9.20	26

TABLE 61

RELATION OF PHOSPHATE BUFFER TO THE PRECIPITATION IN A 5% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI BEFORE AND AFTER HEATING AT 115 (±1)° C. FOR 30 MINUTES

cc. 0.1 M NaH_2PO_4	cc. 0.1 M Na_2HPO_4	cc. 15 per cent $C_{12}H_{11}O_3N_2Na$	Before Heating			After Heating		
			pH at	°C.	Ppt. cc. per 100 cc.	pH at	°C.	Ppt. cc. per 100 cc.*
0	40	20	9.57	24	0	8.70	25	8
4	36	20	8.78	25	0	8.60	24	8
8	32	20	8.82	24	3	8.62	25	4
12	28	20	8.80	25	8	8.68	26	8
16	24	20	8.79	25	9	8.63	26	6
20	20	20	8.80	25	9	8.49	26	6
24	16	20	8.80	25	12	8.64	26	-**
28	12	20	8.79	25	13	8.61	25	-**
32	8	20	8.78	25	13	8.67	25	8
36	4	20	8.76	25	14	8.62	26	8
40	0	20	8.74	25	14	8.62	25	8

*The pH of a solution of Phenobarbital Sodium U. S. P. XI prepared with the use of water instead of the buffer mixtures was 9.70 at 24° C. The volume of precipitate produced by such a solution heated under these conditions was 10 cc./ 100 cc.

**Indefinite results due to separation of a liquid and to formation of crystals during centrifuging.

cc. showed no precipitate immediately after removal from the autoclave. Although some precipitate was formed after cooling, the amount was less than originally present. This indicated that a lowering of the pH may have decreased the amount of deterioration. Inasmuch as none of the solutions were free of precipitate, such solutions were not considered satisfactory for any practical use.

2. The Use of Sodium Carbonate

An attempt was made to regulate the pH of a solution of phenobarbital sodium by employing various amounts of sodium carbonate above and below the amount necessary to neutralize the amount of phenobarbital equivalent to that theoretically present in a 5% solution of phenobarbital sodium.

Inasmuch as 25-cc. portions were to be placed in four-ounce, glass-stoppered, pyrex bottles, the amount of Phenobarbital U. S. P. XI, which was taken for each portion, was equivalent to the theoretical amount of phenobarbital in 25 cc. of a 5% w/v solution of phenobarbital sodium. Therefore 1.142 Gm. of Phenobarbital U. S. P. XI was accurately weighed and because of stickiness of the material, transferred to the bottle with the aid of 96% ethyl alcohol. The alcohol was evaporated off in a water bath. To determine whether or not any decomposition had taken place under these conditions, other samples were subjected to the same treatment and the melting point of the residues was determined. The melting point was found to be 177.5° C. The melting point of the original drug was the same. Inasmuch as this approximates the highest melting point given by the U. S. P. XI (70), it was felt that no significant decomposition had taken place.

The sodium carbonate employed was purchased in the form of the anhydrous analytical reagent from the Mallinckrodt Chemical Works. In conducting the experiment, various amounts of sodium carbonate were added in the form of a 2.6% w/v solution to 1.142-Gm. portions of Phenobarbital U. S. P. XI contained in pyrex bottles. The amount of sodium carbonate required to neutralize this amount of phenobarbital is 0.521 Gm. The water added was equivalent to the difference between 25 cc. and the volume of sodium carbonate solution used. Several samples thus prepared were assayed by the Budde method. Sufficient sodium carbonate was added to bring its concentration up to the level employed in this assay. The remainder of the assay was conducted as previously described. Before heating, the mixtures were shaken mechanically for one hour. A small amount of phenobarbital remained undissolved in each bottle. However solution was brought about in each case because every solution was clear upon removing from the autoclave. The bottles were immediately immersed in a mixture of ice and water. The samples were assayed in the same manner that the controls were assayed.

The results obtained are summarized in Table 52. Each figure given in the table is an average of two determinations.

Although the amounts of sodium carbonate added were above and below the amount necessary to neutralize the amount of phenobarbital present, the pH of the mixtures remained at pH 9.0. The pH of each sample was lowered during treatment. The pH fell from pH 9.0 to pH 8.8 in those samples which had an excess of sodium carbonate. The pH fell from 9.0 to pH 8.7 in those samples which had an insufficient amount of sodium carbonate to neutralize the amount of phenobarbital present. The deteriora-

TABLE 52

EFFECT OF SODIUM CARBONATE ON THE DETERIORATION OF AN APPROXIMATELY 5% W/V SOLUTION OF PHENOBARBITAL SODIUM* HEATED AT 115 (± 1)° C. FOR THIRTY MINUTES

Gm. Na_2CO_3**	pH at 24° C. before Heating	pH at 24° C. after Heating	% $C_{12}H_{11}O_3N_2Na$	% Deterioration
0.55	9.02	8.81	4.18	14.9
0.54	9.02	8.83	4.20	14.5
0.53	9.00	8.77	4.20	14.5
0.52	9.00	8.77	4.21	14.3
0.51	9.02	8.74	4.23	13.9
0.50	9.02	8.72	4.24	13.7
0.49	9.00	8.69	4.27	13.0
0.48	9.00	8.72	4.29	12.6

*Assays of unheated solutions indicated that the amount of phenobarbital present was equivalent to the theoretical amount of phenobarbital in 25 cc. of a 4.01% solution of phenobarbital sodium.

**The amount of anhydrous sodium carbonate A. R. mentioned above was added in the form of a 2.5% w/v solution to 1.142 Gm. of Phenobarbital U. S. P. XI. The volume of water added was equivalent to the difference between 25 cc. and the volume of sodium carbonate solution used.

tion was somewhat less in the latter solutions. A satisfactory decrease in deterioration was not obtained by the use of sodium carbonate in connection with phenobarbital.

C. EVALUATION OF SEVERAL SUGGESTED STABILIZING AGENTS ON SOLUTIONS OF PHENOBARBITAL AND PHENOBARBITAL SODIUM

1. An Experiment on the Stability of Elixir of Phenobarbital U. S. P. XII and Several Modifications.

Elixir of Phenobarbital U. S. P. XII (71) represents the only liquid preparation of phenobarbital which is official. It was decided to investigate its stability and the stability of several modified forms of this solution through the agency of the Budde Assay.

The several formulas studied are as follows:

Formula A

Elixir of Phenobarbital U. S. P. XII

Phenobarbital	0.4 Gm.
Tincture of Sweet Orange Peel	5. cc.
Solution of Amaranth	1. cc.
Alcohol, 96% v/v	12.6 cc.
Glycerin	45. cc.
Syrup	15. cc.
Water, distilled q. s. ad	100. cc.

Formula B

Phenobarbital	0.4 Gm.
Alcohol, 96% v/v	14.9 cc.
Glycerin	45. cc.
Syrup	15. cc.
Water, distilled q. s. ad	100. cc.

Formula C

Phenobarbital	0.4 Gm.
Alcohol, 96% v/v	14.9 cc.
Glycerin	45. cc.
Water, distilled q. s. ad	100. cc.

Formula D

Phenobarbital	0.4 Gm.
Methenamine	0.4 Gm.
Alcohol, 95% v/v	14.9 cc.
Glycerin	45. cc.
Syrup	15. cc.
Water, distilled q. s. ad	100. cc.

The amount of 95% v/v alcohol in the formulas devoid of the tincture was adjusted to maintain the same concentration of alcohol as occurs in the official elixir. For the sake of better control, the equivalent amounts of alcohol, glycerin, and syrup were weighed instead of measured. Methenamine U. S. P. XI was employed in Formula D because it was claimed by Eli Lilly and Company (39) that stable solutions of amytal could be made by dissolving amytal in hydroalcoholic mixtures containing a sufficient quantity of methenamine. The amount of methenamine employed was equivalent to the concentration of the barbiturate.

In a previous experiment it was found that the Budde method, as described before, could be employed in assaying phenobarbital dissolved in such solvents as 95% ethyl alcohol and Glycerin U. S. P. XI. Accurate results were not obtained when the phenobarbital was dissolved in an aqueous solution of Methenamine U. S. P. XI. In the latter case the appearance of the turbidity in the titration was gradual, making the end point uncertain.

Four, 35-cc. portions of each of the above solutions were placed in four-ounce, glass-stoppered, pyrex bottles. Two bottles of each solution were heated for each of two periods. The conditions were 15 minutes at 100 ($\pm$ 0.5)° C. and 30 minutes at 115 ($\pm$ 1)° C. The Budde assay was conducted in the manner previously described with the exception that

25-cc. portions were withdrawn from each bottle and pipetted into a solution of the required amount of sodium carbonate in 25 cc. of water. The averages of the results obtained on assaying solutions prepared according to formulas B and C are indicated in Table 53.

TABLE 53

THE STABILITY OF MODIFICATIONS OF ELIXIR OF PHENOBARBITAL, U. S. P. XII

Formula	B	C
Before Heating		
% $C_{12}H_{11}O_3N_2Na$ in solution	0.399	0.402
After Heating at 100 (± 0.5)° C. for 15 minutes		
% $C_{12}H_{11}O_3N_2Na$ in solution	0.399	0.399
% Deterioration	0.0	0.5
After Heating at 115 (± 1)° C. for 30 minutes		
$C_{12}H_{11}O_3N_2Na$ in solution	0.400	0.400
% Deterioration	0.0	0.5

An attempt was made to assay the official elixir before and after the periods of heating. Satisfactory results could not be obtained because a darkening of the solution was produced due to the action of the sodium carbonate on the amaranth. In addition to the resultant decrease in light intensity of the ray of light passing through the solution under which conditions the end point is observed, the solution was turbid before the addition of silver nitrate solution. Assay of the solution

containing methenamine was also unsuccessful because of the gradual appearance of a turbidity during the titration as previously encountered when assaying in the presence of methenamine. However no precipitate was produced in any of the solutions after heating for the two periods mentioned. This did not necessarily indicate a stabilizing action because of possible solubility of the decomposition products in alcohol. The Budde assay of the solutions differing from the official elixir in the deletion of the tincture and the solution of amaranth indicated no deterioration after both periods of heating. Approximately 1% deterioration after both periods of heating was indicated in the solutions which differed from the official elixir in that the tincture, solution of amaranth, and syrup were deleted.

2. An Experiment on the Stability of Phenobarbital Sodium U. S. P. XI in the Presence of Alcohol, Glycerin, and Antipyrine.

To continue the investigation of the value of alcohol and glycerin as stabilizing agents, an experiment on the stability of 10% w/v solutions of Phenobarbital Sodium U. S. P. XI in the presence of alcohol and glycerin was conducted.

Baldi (68) claimed that a stable solution of phenobarbital sodium could be made by dissolving it in a mixture of equal parts of glycerin and water. Formula C given below was stated by Baldi (68) to be a stable solution. Formula E was modeled after a formula designed by Hazelton, Koppanyi, and Linegar (54). The following formulas were selected in studying the effect of alcohol and glycerin, alone and in combination:

Formula A

Phenobarbital Sodium	10 Gm.
Alcohol, 95% v/v	50 Gm.
Water, distilled q. s. ad	100 cc.

Formula B

Phenobarbital Sodium	10 Gm.
Glycerin	50 Gm.
Water, distilled q. s. ad	100 cc.

Formula C

Phenobarbital Sodium	10 Gm.
Glycerin	20 Gm.
Alcohol, 95% v/v	10 Gm.
Water, distilled q. s. ad	100 cc.

Formula D

Phenobarbital Sodium	10 Gm.
Glycerin	10 Gm.
Alcohol, 95% v/v	10 Gm.
Water, distilled q. s. ad	100 cc.

Formula E

Phenobarbital Sodium	10 Gm.
Glycerin	10 Gm.
Alcohol, 95% v/v	10 Gm.
Antipyrine	10 Gm.
Water, distilled q. s. ad	100 cc.

A 25% w/v solution of Phenobarbital Sodium U. S. P. XI was prepared from a lot of drug purchased with the Mallinckrodt control designation KEM. From this solution all of the other solutions were prepared. Into the pyrex bottles previously mentioned in preceding experiments were added 35-cc. portions of these solutions. Some were heated at 100 ($\pm$ 0.5)° C. for 15 minutes and others were heated at 115 ($\pm$ 1)° C. for 30 minutes. In conducting the Budde assay, 25 cc. of the solution was withdrawn from each bottle and 8.33 Gm. of anhydrous sodium carbonate was added, followed by

sufficient water to make 250 cc. Of the dilution, 25 cc. was taken for the titration. The results are summarized in Table 54. Each figure given in this table represents the average of two determinations.

From the results given in Table 54, it appeared that a 10% w/v solution of Phenobarbital Sodium U. S. P. XI in approximately 40% w/v alcohol is more stable at 115° C. than solutions containing glycerin alone or glycerin and alcohol in the combinations and percentages studied. The solution containing 40% w/v alcohol showed 1.5% deterioration after heating at this temperature for 30 minutes, whereas the other solutions similarly treated showed approximately 8% deterioration which was only slightly less than that shown by the control solution of 10% w/v Phenobarbital Sodium U. S. P. XI in water alone. Somewhat less deterioration than in the control was shown by all of the formulas mentioned in the table when heated at 100° C. for 15 minutes.

The solution containing the antipyrine could not be assayed by the Budde method inasmuch as the results obtained with the untreated solution were inconsistent and were in marked difference with the untreated control solution. Although this solution formed no precipitate after heating at the shorter period of time and only approximately 1 cc. of precipitate per 100 cc. after heating at the longer period of time, no conclusion was drawn concerning its stability because of the possible solution of the decomposition products in the alcohol present.

3. An Experiment on the Stability of Solutions of Phenobarbital Sodium U. S. P. XI containing Dextrose.

An experiment was conducted to learn whether or not a 10% w/v solution

TABLE 64

THE STABILITY OF A 10% W/V SOLUTION OF PHENOBARBITAL SODIUM U. S. P. XI IN THE PRESENCE OF GLYCERIN, ALCOHOL, AND ANTIPYRINE

Formula	Control	A	B	C	D
Before Heating					
% $C_{12}H_{11}O_3N_2Na$ in solution	9.76*	9.75	9.75	9.76	9.77
Ppt. cc./100 cc.	0	0	0	0	0
After Heating at 100 (± 0.5)° C. for 15 minutes					
% $C_{12}H_{11}O_3N_2Na$ in solution	9.51	9.62	9.61	9.61	9.66
% deterioration	2.6	1.5	1.4	1.6	1.1
Ppt. cc./100 cc.	1	0	0	1	1
After Heating at 116 (± 1)° C. for 30 minutes					
% $C_{12}H_{11}O_3N_2Na$ in solution	8.88	9.60	8.96	9.02	8.96
% deterioration	9.0	1.5	8.1	7.6	8.3
Ppt. cc./100 cc.	9	0	4	6	1

*The amount of phenobarbital sodium in the calculated dry sample used to make this solution was accordingly 97.6%.

of phenobarbital sodium could be stabilized by replacing a portion of the solvent with dextrose.

Several formulas containing 50% w/v Dextrose U. S. P. XI in the presence of water, alcohol, and glycerin were studied. The Phenobarbital Sodium U. S. P. XI was purchased with the Mallinckrodt control designation XXX.

Formula A

Phenobarbital Sodium		10 Gm.
Dextrose		50 Gm.
Water, distilled	q. s. ad	100 cc.

Formula B

Phenobarbital Sodium		10 Gm.
Dextrose		50 Gm.
Alcohol, 95% v/v		10 Gm.
Water, distilled	q. s. ad	100 cc.

Formula C

Phenobarbital Sodium		10 Gm.
Dextrose		50 Gm.
Glycerin		10 Gm.
Water, distilled	q. s. ad	100 cc.

The manner in which these solutions were treated and assayed was the same as that described in the preceding experiment. The results are given in Table 55. The figures represent the average of two determinations.

From Table 55, it can be seen that only the 10% w/v solution containing approximately 9% w/v ethyl alcohol indicated any stabilizing action at all. However its action was too slight to be conclusive. All of the solutions became colored brown after heating at 100° C. for 15 minutes and after heating at 115° C. for 30 minutes. The solutions here mentioned were considered unsatisfactory because of this coloration and lack of

TABLE 55

THE STABILITY OF 10% W/V SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI CONTAINING 50% W/V DEXTROSE U. S. P. XI

Formula	Control	A	B	C
Before Heating				
% $C_{12}H_{11}O_3N_2Na$ in solution	9.74*	9.75	9.71	9.75
After Heating at 100 (± 0.5)° C. for 16 minutes				
% $C_{12}H_{11}O_3N_2Na$ in solution	9.51	8.87	9.54	9.04
% deterioration	2.4	9.0	1.8	7.1

*The amount of phenobarbital sodium in the calculated dry sample used to make the solution was accordingly 97.4%.

stability.

IV. DISCUSSION OF RESULTS

The present investigation was concerned with a study of a number of factors in the deterioration of solutions of phenobarbital sodium, together with research on several suggested methods of retardation of this process. Methods of assay applicable to the problem were compared.

Methods of Assay

The hygroscopic nature of phenobarbital sodium necessitated a method of determining the percentage moisture present. Comparison of results obtained on drying samples at temperatures slightly above 100° C. and at temperatures slightly above 140° C. showed 1.7% and 3.4% loss in weight respectively. Vacuum desiccation at room temperature revealed a loss in weight of 1.6%, which is a value approximating the loss sustained at the former temperature. Budde assays did not reveal any decrease in the amount of phenobarbital sodium originally present in the lot of drug employed, regardless of which of the two temperatures were used in drying.

Numerous methods of assay were reported in the literature. Certain methods were selected as worthy of study. Reasons for not investigating the other methods have already been given. The lot of Phenobarbital Sodium U. S. P. XI employed conformed to the official purity rubric inasmuch as 91% "phenobarbital" was extracted by means of the official assay. By means of the modified and unmodified Rotondaro assays, 75.2% and 78.6% phenobarbital respectively was extracted from samples of the same lot of drug. The average sum of the three residues obtained in

these Rotondaro assays was below the U. S. P. XI tolerance. The number of treatments with chloroform to extract each residue in the modified Rotondaro assay was increased nearly twofold. The sum of the three residues was again found to be below the U. S. P. XI tolerance for "phenobarbital". Modified Rotondaro assays of Phenobarbital U. S. P. XI and of phenobarbital previously recovered in other Rotondaro assays gave an average recovery of phenobarbital more than 20% below the original amount taken. Moreover the sum of the three residues only averaged 91.7%.

Several methods of assay employing silver nitrate were reported but all are exemplified by the Budde (98), Viebock and Fuchs (95, 96), and Shulek and Kossa (100, 101) methods. These methods of assay showed respectively 89.6%, 89.1%, and 85.4% phenobarbital present in the lot of Phenobarbital Sodium U. S. P. XI employed. The U. S. P. XII tolerance is 99.0 to 91.6% phenobarbital. The end points in the latter two methods were difficult to determine when assaying this drug.

The chemistry of the Budde assay was investigated. Silver in the form of silver barbiturate, dissolved in a solution containing sodium carbonate, was not precipitated upon the addition of sodium chlorides. The compound formed on adding silver nitrate to phenobarbital was insoluble in water but soluble in the presence of sodium carbonate. Addition of phenobarbital to a suspension of silver carbonate containing excess sodium carbonate resulted in a clear solution.

The possible interference of decomposition products in the Budde assay was investigated. No significant error was revealed on titrating a solution of phenylethylacetylurea nor on titrating a solution of phenylethylacetylurea which had been heated under the most drastic conditions

to which solutions in subsequent experiments were subjected. The maximum breakdown possible in the investigation was thus duplicated to assure formation of phenylethylacetic acid and urea.

The von Babitsch titration (66) of pure phenobarbital using standard alkali in the presence of thymolphthalein gave an average result of 99.8%.

In a comparison of various assays on a deteriorated solution of phenobarbital sodium, the U. S. P. XI assay showed 8.7% deterioration in a 4% w/v solution of Phenobarbital Sodium U. S. P. XI heated at 115° C. for 30 minutes. The modified Rotondaro assay showed 34.7% deterioration and the Budde assay showed 10.6% deterioration in the same solution.

Effect of Heating Solutions of Phenobarbital Sodium U.S.P. XI

Solutions of Phenobarbital Sodium U. S. P. XI in concentrations of 5, 10, and 20% were subjected to 15, 30, 60, and 120-minute periods of heating at 60°, 80°, 100°, 115°, and 127° C. These conditions were selected to encompass most of the conditions previously employed by other workers and to include, for the most part, the official conditions of sterilization recommended for solutions intended for parenteral use. The results are summarized in Table 56. A comparison of these results with the results obtained by Tomaki and Waller (34) under some of these conditions is afforded by Table 57.

By the results given in Table 56, it was indicated that sterilization of solutions through the agency of heat is accompanied by deterioration, the extent of which is dependent upon temperature and duration of exposure. Hesa (109) reported that moist heat at 80° C. for 60 minutes kills all living bacteria but not their spores. The latter are killed in

TABLE 56

EFFECT OF HEATING SOLUTIONS OF PHENOBARBITAL SODIUM U. S. P. XI*

	60° C.	80° C.	100° C.	115° C.	127° C.
			5% w/v Solution		
0 min.	9.51	9.53	9.53	9.48	9.60
15 min.	0.0 9.51 0	0.4 9.60 0	2.1 9.43 0	5.9 9.22 2	13.9 8.81 5
30 min.	0.0 9.49 0	1.0 9.48 0	4.1 9.23 2	12.7 8.92 4	23.6 8.86** 9
60 min.	2.0 9.43 0	1.6 9.37 0	10.1 9.01 4	21.9 8.68 5	37.1 8.44** 10
120 min.				37.0 8.62 10	56.6 8.16** 17
			10% w/v Solution		
0 min.		9.63	9.62	9.62	9.73
15 min.		0 9.60 0	2.1 9.41 0	5.2 9.28 4	11.3 9.24 10
30 min.		0.4 9.56 0	5.1 9.23 4	11.1 8.92 10	22.6 8.73** 16
60 min.		1.9 9.41 1	9.0 9.05 6	22.3 8.99** 15	36.8 8.51** 24
120 min.				35.0 8.93** 16	53.6 8.63** 27

*The sets of three figures after each period and below a particular temperature represent in order: a) per cent deterioration, b) pH, and c) volume of precipitate, cc./ 100 cc. Blank spaces indicate that determinations were not made. After " 0 min." only the pH of the original solution is given.

**Ammonia was detected.

TABLE 56 (continued)*

	60° C.	80° C.	100° C.	115° C.	127° C.
			20% w/v Solution		
0 min.		9.71	9.80	9.76	9.91
15 min.		0.8 9.69 0	2.1 9.61 1	6.2 9.35 4	11.8 9.49 13
30 min.		1.5 9.66 0	6.3 9.38 5	11.0 9.12 11	22.3 9.29** 17
60 min.		3.0 9.51 3	9.6 9.16 14	21.2 8.88 32	37.1 9.10** 35
120 min.				35.6 8.61 43	54.0 8.36**

*The sets of three figures after each period and below a particular temperature represent in order: a) per cent deterioration, b) pH, and c) volume of precipitate, cc./ 100 cc. Blank spaces indicate that determinations were not made. After "0 min." only the pH of the original solution is given.

**Ammonia was detected.

TABLE 67

PERCENTAGE DETERIORATION FOUND BY TOMSKI AND WALLER ON HEATING 20% SOLUTIONS OF PHENOBARBITAL SODIUM CONTAINED IN AMPULS

	80° C.	100° C.	115° C.
15 minutes	-	1.0	-
30 minutes	1.0 to 1.5	-	19.2 to 20.4
60 minutes	2.0 to 3.0	9.5 to 10.5	-

30 minutes at 115° C., according to Huss (109). By the results given in Table 56, it is indicated that such sterilization is accompanied by a small amount of deterioration under the milder treatment mentioned and considerable deterioration under the stronger treatment mentioned.

The results obtained on heating 20% solutions corresponded for the most part to the results obtained by Tomski and Waller (24) under some of these conditions. Differences were noted in percentage deterioration on heating 20% solutions at 100° C. for 15 minutes and at 115° C. for 30 minutes. Tomski and Waller (24) reported 1.0% and 19.2 to 20.4% deterioration under the respective conditions. The results of the present study were 2% and 11% deterioration respectively.

The Budde assay was employed exclusively in determining the percentage deterioration in this study of the effect of heating solutions of Phenobarbital Sodium U. S. P. XI. The amount of phenobarbital sodium in the calculated dry sample used to prepare each solution was determined from the assays on the unheated solution. The average of all the results on per cent of phenobarbital sodium in the calculated dry sample was 97.7% with an average difference between assays of 0.8%. These figures apply to both lots of Phenobarbital Sodium U. S. P. XI used in the present study.

Relation of pH to Deterioration

Upon lowering the pH of a 5% w/v solution of Phenobarbital Sodium U. S. P. XI by means of various amounts of monosodium and disodium phosphates, it was found that phenobarbital was precipitated at pH 8.8, the amount of precipitate increasing with increase in the amount of monosodium phosphate added. Five per cent solutions buffered at pH 8.6 to

8.7 with 12 to 14 cc. of precipitate per 100 cc., showed 6 to 8 cc. of precipitate per 100 cc. after heating at 115° C. for 30 minutes, whereas a solution of the same strength in water alone showed 10 cc. precipitate per 100 cc. after similar heating. Five per cent solutions buffered at the same pH but with less monosodium phosphate showed no marked decrease in amount of precipitate present before and after heating.

Various amounts of sodium carbonate were added to phenobarbital, above and below the amount necessary for neutralization. The amounts of sodium carbonate added varied between a maximum of 5.6% above and a minimum of 7.7% below the amount of sodium carbonate necessary to neutralize the amount of phenobarbital present in an equivalent volume of 5% solution of phenobarbital sodium. The pH of the mixtures remained at pH 9.0 with the quantities of sodium carbonate used. After heating at 115° C. for 30 minutes, the pH fell from pH 9.0 to pH 8.6 in those samples which had an excess of sodium carbonate. The pH fell from pH 9.0 to pH 8.7 in those samples which had an insufficient amount for neutralization. The per cent deterioration under these conditions ranged from 14.9 to 12.6%, being lower in the solutions having the smaller amounts of sodium carbonate.

Evaluation of Several Suggested Stabilising Agents.

The value of several suggested stabilising agents was investigated. Elixir of Phenobarbital U. S. P. XII, with and without 0.4% methenamine, could not be assayed by the Budde method, but no precipitate was evident after heating at 100° C. for 15 minutes and after heating at 115° C. for 30 minutes. The solutions differing from the official elixir in the deletion of the tincture and the solution of amaranth showed no deterioration

after both periods of heating. Approximately 1% deterioration after both periods of heating was indicated in the solution which differed from the official elixir in that the tincture, solution of amaranth, and syrup were deleted.

Results were obtained which indicated that a 10% w/v solution of Phenobarbital Sodium U. S. P. XI in approximately 46% w/v ethyl alcohol is more stable at 115° C. than: a) a solution containing 50% w/v glycerin, b) a solution containing 20% w/v glycerin and 9% w/v ethyl alcohol, and c) a solution containing 10% w/v glycerin and 9% w/v ethyl alcohol. The solution containing 46% w/v ethyl alcohol showed 1.8% deterioration after heating under the latter mentioned conditions, whereas the other solutions similarly treated showed approximately 8% deterioration, the latter figure being only slightly less than shown by the control solutions of 10% w/v Phenobarbital Sodium U. S. P. XI in water alone. Somewhat less deterioration than in the control was shown by all of the solutions when heated at 100° C. for 15 minutes. The solution containing 10% w/v each of antipyrine and glycerin and 9% w/v ethyl alcohol could not be assayed but formed no precipitate after heating under the latter mentioned conditions and only 1 cc. of precipitate per 100 cc. after heating at 115° C. for 30 minutes, whereas the control showed respectively 1 cc. and 9 cc. of precipitate per 100 cc. under the same conditions.

Solutions containing 10% w/v Phenobarbital Sodium U. S. P. XI in the presence of 50% w/v dextrose, with or without either 9% w/v ethyl alcohol or 10% w/v glycerin turned brown after heating at 100° C. for 15 minutes and after heating at 115° C. for 30 minutes. The solution containing 9% w/v ethyl alcohol and 50% w/v dextrose showed slightly less

percentage deterioration than a control solution of 10% w/v solution of Phenobarbital Sodium in water alone.

V. SUMMARY AND CONCLUSIONS

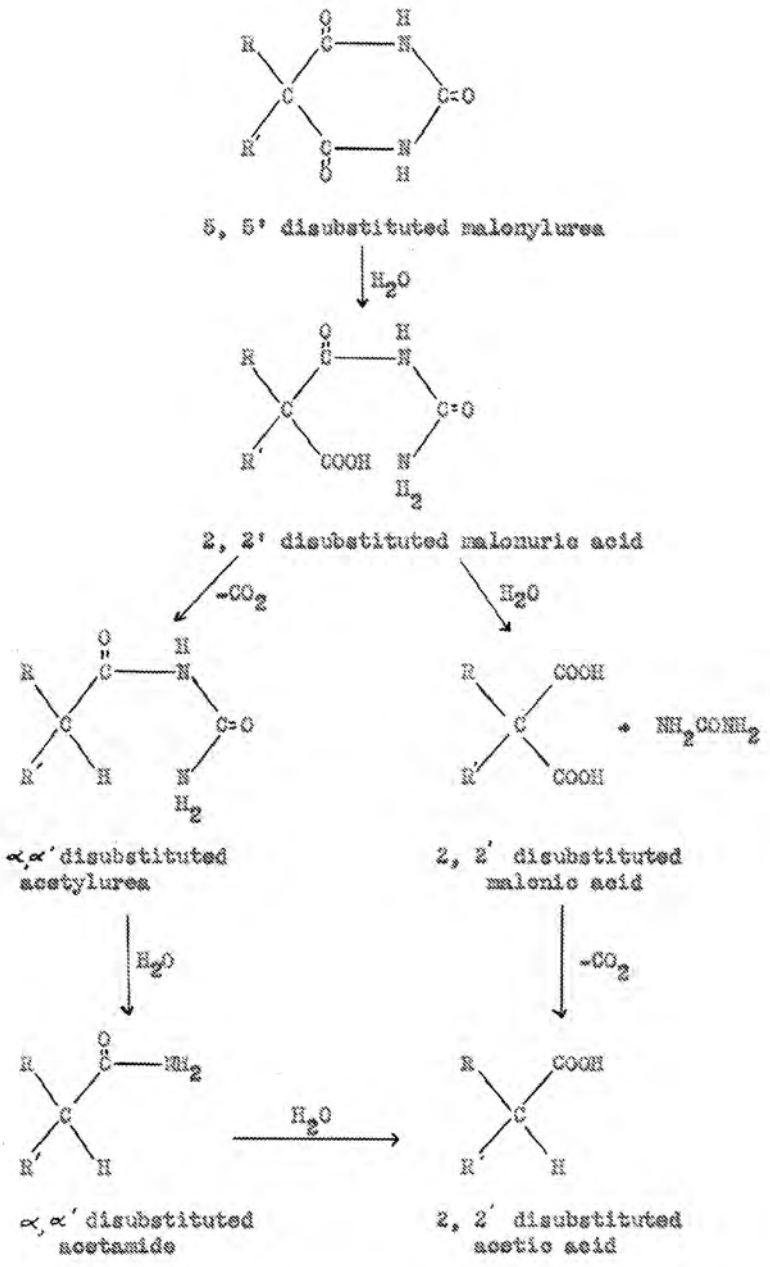
Nature of the Deterioration

The products formed during the decomposition of barbiturate solutions and the manner in which they are produced were revealed by several workers, namely Steenhauer (2), Madsen (3), Bailey (4), Nielsen (10, 11, 13) and Aspelund and his coworkers (7, 8, 9). Their findings may be summarized by the outline which appears on the following page. From the agreement in the results reported by the aforeto mentioned workers, the nature of the decomposition appeared to be amply explained.

An investigation was carried out on a number of factors, such as time, temperature, and concentration, which are involved in the deterioration of solutions of phenobarbital sodium. The effects produced in pH, volume of precipitate, and refractive index had not been reported for this compound under the conditions of the present study. In addition an extension of the work on percentage deterioration performed by Tomaki and Waller (24) was made.

Methods of Assay

Methods of determining percentage moisture in Phenobarbital Sodium U. S. P. XI were studied and compared. Comparison of results obtained on vacuum desiccation with those obtained on heating at 100° C. and 140° C. indicated either incomplete drying at 100° C. or the presence of decomposition at 140° C. Budde assays of samples heated at either temperature


5, 5' disubstituted malonylurea
H_2O
2, 2' disubstituted malonuric acid
$-CO_2$
H_2O
$+ NH_2CONH_2$
α,α' disubstituted acetylurea
2, 2' disubstituted malonic acid
H_2O
$-CO_2$
α,α' disubstituted acetamide
H_2O
2, 2' disubstituted acetic acid

failed to show any decomposition. However, it was deemed advisable to avoid the introduction of an error due to possible deterioration which might have been undetected in samples heated at 140° C. Undried samples of known moisture content were generally used in the investigation.

Numerous methods of assay of barbiturates were found in the literature. Nielsen (13) and Madsen (3) calculated the percentage deterioration by determining the amount of dialkylacetylurea formed. This appeared to be at fault because of possible further decomposition of dialkylacetylurea which would consequently lead to lower results as revealed by Schlemmer and Törber (30). The methods employing an immiscible solvent at a single pH level were considered unsatisfactory because of possible simultaneous extraction of decomposition products. The methods in which alkalinity or acidity of the barbiturate derivative was measured by simply titrating with a standard acid or alkali were not applicable because of the existence of alkaline and acidic decomposition products in deteriorated solutions. Colorimetric methods were not investigated because of their reported inaccuracies.

Experiments were conducted on the modified and unmodified Rotondaro assays and the U. S. P. XI assays of Phenobarbital Sodium U. S. P. XI. Both types of Rotondaro assays failed markedly in duplicating the amount of phenobarbital recovered by the U. S. P. XI assay. This indicated the existence of several possibilities: a) the original sample was partially decomposed, b) decomposition took place during the modified and unmodified Rotondaro assays, c) incomplete extraction, and d) failure of the assay to separate the decomposition products quantitatively without admixture. Increase in the number of shakings-out with chloroform failed

to produce a recovery of phenobarbital comparable to that obtained in the U. S. P. XI assay. Therefore modified Rotondaro assays were carried out on samples of Phenobarbital U. S. P. XI and samples of phenobarbital previously obtained from other Rotondaro assays. The amount of phenobarbital recovered was greatly below the amount taken. The presence of possibilities b, c, and d were indicated. Rotondaro (72) claimed that 98% phenobarbital could be recovered by means of his assay.

Several methods of assay employing silver nitrate were compared. The methods designated by Vieböck and Fuchs (95, 96) and by Shulek and Rossa (100, 101) were not considered applicable because of difficulty experienced in determining the end point when assaying phenobarbital sodium. The Budde assay yielded results within the U. S. P. XII tolerance. The chemistry of the Budde assay was investigated. In support of the claims made by the originator, results were obtained which indicated that a silver derivative of phenobarbital is formed during the assay which is soluble in the presence of sodium carbonate and which is weakly dissociated in this media. Additional tests indicated that up to the end point the addition of silver nitrate to a solution of phenobarbital sodium in the presence of sodium carbonate is not accompanied by the formation of silver carbonate or silver oxide but rather by the formation of a silver barbiturate derivative. This appeared to be explained by removal of silver ions from the field of reaction due to their combination with the barbiturate to form a weakly dissociated compound.

Experiments were performed to learn whether or not the presence of decomposition products would interfere with the Budde assay. The titer

of a saturated solution of phenylethylacetylurea was found to be so low that no significant error would be introduced. Similar results were obtained on titrating a solution of phenylethylacetylurea which had been heated to produce the maximum amount of phenylethylacetic acid and urea possible under the conditions of the present investigation. The Budde assay yielded consistent results in the numerous assays which were performed during the experiments on the effect of heat on solutions of phenobarbital sodium. Here the average of all the results on per cent of phenobarbital sodium in the calculated dry sample used to prepare each solution was 97.7%, with an average difference between assays of 0.3%. These figures apply to both lots of Phenobarbital Sodium U. S. P. XI used in the present study.

A deteriorated solution of phenobarbital sodium was assayed by the modified Rotondaro method, the U. S. P. XI method and the Budde method. The results differed widely. The U. S. P. XI yielded results which were too low because the decomposition products and the phenobarbital are extracted as one. The results yielded by the modified Rotondaro assays were concluded to be too high because it had been previously indicated that the recovery of phenobarbital was incomplete in this assay. It was concluded that the Budde method gives results that are approximately correct or that are sufficiently accurate for determining the relative degree of deterioration in comparative tests.

Effect of Heating Solutions of Phenobarbital Sodium U. S. P. XI

The effect of such factors as time, temperature, and concentration were studied with regard to the per cent deterioration, pH change, change

in refractive index, and volume of precipitate which occurred in solutions of phenobarbital sodium. The temperature and periods of exposure were selected to encompass most of the conditions previously employed by other workers and to include, for the most part, the official conditions of sterilization recommended for solutions intended for parenteral use. An insight into the deterioration which might take place during prolonged storage was afforded by means of these accelerated tests. It was found that an increase in temperature was accompanied by a drop in pH, an increase in amount of precipitate, and an increase in percentage deterioration on comparing solutions of equal concentration, heated for one period of time. Keeping the temperature constant and increasing the period of exposure produced similar changes. Madsen (3) found an increase in pH-drop and an increase in percentage deterioration on heating 10% solutions of barbital sodium. Cazzani (10) reported that solutions of amytal sodium displayed a decrease in pH on aging. Bailey (4) found that greater amounts of precipitate were formed on increasing the temperature of solutions of barbital sodium and on prolonging the period of heating. Keeping the temperature and the time constant, the percentage deterioration generally showed little change on increase in concentration of the original solution under the conditions studied. This was in agreement with the findings of Tomaki and Waller (24). As might be expected, the refractive index underwent a decrease with each increase in amount of precipitate.

Relation of pH to Deterioration of Solutions of Phenobarbital Sodium

From a study on the deterioration of solutions of Phenobarbital Sodium U. S. P. XI, it was found that the pH levels of 5, 10, and 20% w/v solutions of this drug were pH 9.6, 9.7, and 9.8 respectively. Berassin and Vitali (28) stated that the pH of a 10% solution was 9.6. No mention was found in the literature of the pH at which phenobarbital is precipitated from solutions of phenobarbital sodium. Upon adding various amounts of phosphate buffer to a 5% solution, a precipitate was first noted at pH 8.8. In confirmation of the findings of Berassin and Vitali (28), it was found that a decrease in amount of precipitate was evident on heating solutions which had a lower pH than an unbuffered solution. Likewise Nielsen (13) stated that the rate of hydrolysis of solutions of phenobarbital sodium was decreased when there was an increase in hydrogen ion concentration. The decrease in the amount of precipitate formed on heating solutions buffered with monosodium and disodium phosphate was not sufficient to warrant a conclusion that any marked stabilization had been brought about. The use of sodium carbonate in amounts insufficient to completely neutralize the amount of phenobarbital resulted in mixtures of lower pH. The per cent deterioration was only slightly decreased thereby.

Value of Several Suggested Stabilizing Agents

Experiments were conducted on the stability of Elixir of Phenobarbital U. S. P. XI and several of its modifications. Little or no deterioration was found on heating solutions which differed from the official elixir in the deletion of the tincture and the solution of amaranth or in solutions

differing in the deletion of the two aforeto mentioned ingredients together with the syrup. The conditions of heating were 100° C. for 15 minutes and 115° C. for 30 minutes. Although the percentage deterioration of the official elixir, with and without 0.4% methenamine, could not be determined by the Budde assay, no precipitate was evident after subjecting the preparations to the conditions mentioned. However no definite conclusion was drawn concerning their stability because of the possible solution of the decomposition products in the alcohol present. Eli Lilly and Company (39) claimed that stable solutions of amytal could be made by dissolving the drug in hydroalcoholic mixtures containing methenamine. The percentage deterioration of a 10% w/v solution of phenobarbital sodium containing 48% w/v ethyl alcohol showed markedly less per cent deterioration than that shown by solutions containing 50% w/v glycerin or solutions containing alcohol and glycerin. These solutions were heated at 115° C. for 30 minutes. Baldi (88) stated that stable solutions of phenobarbital sodium could be made by dissolving the drug in a mixture of equal parts of glycerin and water. Haselton, Koppanyi, and Linegar (54) employed 10% w/v each of glycerin, alcohol, and antipyrine as stabilizing agents for 10% solutions of the sodium salt of secondary amyl bromallyl barbiturate. A solution of 10% phenobarbital sodium was prepared according to their formula. The Budde assay could not be carried out but it was found that no precipitate was formed after heating at 100° C. for 15 minutes and only approximately 1 cc. precipitate per 100 cc. after heating at 115° C. for 30 minutes. No conclusion was drawn concerning its stability.

The use of 50% w/v dextrose in 10% w/v solutions of phenobarbital sodium was unsatisfactory due to the formation of a brown color on heating at 100° C. for 15 minutes and on heating at 115° C. for 30 minutes.

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BIOGRAPHICAL ITEMS

Bernard S. Jatul was born on January 15, 1918 in Boston, Massachusetts.

Undergraduate Studies. He graduated from the Massachusetts College of Pharmacy in June, 1939 and attended the 1938 summer session at Harvard University.

Graduate Studies. The Master of Science degree was conferred by Purdue University in August, 1940. Mr. Jatul attended the University of Florida from September, 1940 to February, 1943.

Experience. He served as a Graduate Assistant in Pharmacy while pursuing his studies at the University of Florida.

Honors. At the Massachusetts College of Pharmacy he received the McKesson and Robbins Scholarship, the Scholarship of Mu Chapter of Kappa Psi, the Leon C. Ellis Prize, and the Kappa Psi Grand Council Scholarship Key. He served as President of the Iota Chapter of the Rho Chi Society at the University of Florida during 1940-1941.

This dissertation was prepared under the direction of the Chairman of the candidate's Supervisory Committee and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date Feb. 5, 1943

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	Filing Date	2020-09-18
	First Named Inventor	Bala Tripura Sundari Chodavarapu
	Art Unit	1617
	Examiner Name	Yanzhi ZHANG
	Attorney Docket Number	104078.0001US

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	Attorney Docket Number	104078.0001US

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/Y.Z/	1	GUPTA, V. Das, "Effect of Ethanol, Glycerol, and Propylene Glycol on the Stability of Phenobarbital Sodium," Journal of Pharmaceutical Sciences, 1984 November 1; 73(11):1661-1662. Abstract only.	
/Y.Z/	2	NAYYAR et al., "Phenytoin-Folate Interactions: How Far is Safe Folate Supplementation in Phenytoin Treated Epileptic Patients?" Journal of Applied Pharmaceutical Science, 2012; 02(06):230-235.	

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Examiner Signature	/YANZHI ZHANG/	Date Considered	02/17/2021

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EFS Web 2.1.18

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /Y.Z/

Zhang, Yanzhi

From: James, Valencia
Sent: Monday, February 22, 2021 9:09 AM
To: Zhang, Yanzhi
Subject: FW: Confirmation Receipt: Reference Delivery Request - 17/025881

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Valencia James
Systems Information Searcher
US Patent Trademark Office
Reference Delivery Branch
571 272-2517
Valencia.james@uspto.gov

From: Zhang, Yanzhi <Yanzhi.Zhang@USPTO.GOV>
Sent: Monday, February 22, 2021 8:35 AM
To: STIC-ILL <STIC-ILL@USPTO.GOV>
Subject: RE: Confirmation Receipt: Reference Delivery Request - 17/025881

Good morning,
Any update for the following ref. request?

Thanks,

Yanzhi Zhang
Primary Examiner
USPTO, AU 1617
571-272-3117

From: STIC-ILL <STIC-ILL@USPTO.GOV>
Sent: Thursday, February 18, 2021 1:36 PM
To: Zhang, Yanzhi <Yanzhi.Zhang@USPTO.GOV>
Cc: STIC-ILL <STIC-ILL@USPTO.GOV>
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US

CONFIRMATION NO. 8113

149344
Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

PUBLICATION NOTICE

OC000000124368395

Title: Lyophilized Compositions of Phenobarbital Sodium Salt

Publication No.US-2021-0085608-A1
Publication Date:03/25/2021

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 149344

Mar 26, 2021 04:11:26 AM

Dear PAIR Customer:

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
17025881	NTC.PUB	03/25/2021	104078.0001US

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

Electronic Acknowledgement Receipt	
EFS ID:	42717744
Application Number:	17025881
International Application Number:	
Confirmation Number:	8113
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
Customer Number:	149344
Filer:	Martin Fessenmaier/Danielle Moroz
Filer Authorized By:	Martin Fessenmaier
Attorney Docket Number:	104078.0001US
Receipt Date:	13-MAY-2021
Filing Date:	18-SEP-2020
Time Stamp:	16:07:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
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Payment was successfully received in RAM	$680
RAM confirmation Number	E20215CG07593130
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Authorized User	Danielle Moroz
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Continued Examination (RCE)	RCE.pdf	1364301 b21642403a6e2c90e1d7c66115e06c7cb574 0460f	no	3
Warnings:					
Information:					
2		Response-02_RCE_FOA_dated_ 2021-02-26_REV003.pdf	164672 e68720413259d9b847d297c7a42315b8837 5fed2	yes	10
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Applicant Arguments/Remarks Made in an Amendment		5	10	
	Claims		2	4	
	Amendment Submitted/Entered with Filing of CPA/RCE		1	1	
Warnings:					
Information:					
3	Oath or Declaration filed	Response-02-DEC-Signed.pdf	572414 6035574d6c3536973b771dddc5442d12c3 621af9	no	4
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30263 87cec14e36abb07901d67556da31aff0fc90 09ba	no	2
Warnings:					
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Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (02-18)
Approved for use through 11/30/2020. OMB 0651-0031
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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(Submitted Only via EFS-Web)

Application Number	17/025,881	Filing Date	2020-09-18	Docket Number (if applicable)	104078.0001US	Art Unit	1617
First Named Inventor	Bala Tripura Sundari Chodavarapu			Examiner Name	Yanzhi ZHANG		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV

SUBMISSION REQUIRED UNDER 37 CFR 1.114

Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).

☐ Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
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- ☒ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☒ Affidavit(s)/ Declaration(s)
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MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____
(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

× Patent Practitioner Signature

Applicant Signature

EFS - Web 2.1.16

Doc code: RCEX
Doc description: Request for Continued Examination (RCE)

PTO/SB/30EFS (02-18)
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Signature of Registered U.S. Patent Practitioner			
Signature	/Martin Fessenmaier Ph.D./	Date (YYYY-MM-DD)	2021-05-13
Name	Martin Fessenmaier Ph.D.	Registration Number	46697

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

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ALEXANDRIA, VA 22313-1450

Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE TO FINAL OFFICE ACTION

Sir:

Concurrent with the Request For Continued Examination, this paper responds to the Office Action dated 26 February 2021. Please enter the amendments shown herein.

- Claim amendments begin on page 2.
- Remarks begin on page 5.

AMENDMENTS TO THE CLAIMS

This listing of claims represents the current version of claims before the Office:

1. (currently amended) A pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution.
2. (currently amended) A method of producing a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, the method comprising:

   adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.2 to 10.2; and

   lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium.
3. (currently amended) The ~~method~~ pharmaceutical composition of claim 1, wherein the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium is produced by adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.2 to 10.2 and lyophilizing the Phenobarbital Sodium solution ~~2, wherein the water is Water for Injection~~.
4. (currently amended) The ~~method~~ pharmaceutical composition of claim 3, wherein the water is Water for Injection ~~is~~ sparged with nitrogen prior to adding the Phenobarbital Sodium.
5. (currently amended) The ~~method~~ pharmaceutical composition of claim 4, wherein the Water for Injection is sparged with nitrogen for at least 30 minutes prior to adding the Phenobarbital Sodium.
6. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the water is cooled to or between 2 to 8°C prior to adding the Phenobarbital Sodium.
7. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the forming of the Phenobarbital Sodium solution with the pH at or between 9.2 to 10.2 comprises measuring the pH of the Phenobarbital Sodium solution, and wherein if the pH is higher than 10.2, the forming comprises adding Hydrochloric acid (HCl).
8. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.1% phenylethylacetylurea (PEAU)

when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).

9. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.05% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
10. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the lyophilized Phenobarbital Sodium forms no detectable amount of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
11. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the lyophilized Phenobarbital Sodium powder is stable up to at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).
12. (currently amended) The ~~method~~ pharmaceutical composition of claim 11, wherein the stability of the lyophilized Phenobarbital Sodium powder is determined by adding the lyophilized Phenobarbital Sodium powder to an aqueous solution to form a reconstituted ~~lyophilized~~ Phenobarbital Sodium solution and analyzing to quantitate the amount of phenylethylacetylurea (PEAU), 2-ethyl-2-phenylmalonamide (2EPMM), and/or alpha-phenylbutyrylguanidine (PBG) in the reconstituted ~~lyophilize~~ Phenobarbital Sodium solution.
13. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein the Phenobarbital Sodium solution is at a concentration of 65 mg/mL, 100 mg/mL, 130 mg/mL or 200 mg/mL.
14. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising filtering the Phenobarbital Sodium solution prior to lyophilizing.
15. (currently amended) The ~~method~~ pharmaceutical composition of claim 3 ~~2~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising:

aliquoting the Phenobarbital Sodium solution into a vial prior to lyophilization; and

after lyophilization, applying a closure to the vial under vacuum.

16. (currently amended) A method of treating an individual in need of Phenobarbital Sodium, the method comprising:

adding saline or dextrose to a composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium immediately prior to administration to form a Phenobarbital Sodium solution with no more than 0.2% phenylethylacetylurea (PEAU); and

administering the Phenobarbital Sodium solution to the individual,

wherein the efficacy of the treating is not diminished, and the side effects caused by degradation products of the Phenobarbital Sodium are not increased as a consequence of an extended storage duration of the Phenobarbital Sodium powder of at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).

17. (original) The method of claim 16, wherein the individual suffers from epilepsy.

18. (original) The method of claim 17, wherein the individual is a newborn suffering from neonatal epilepsy.

19. (original) The method of claim 16, wherein the composition is a powder dose of 65 mg, 100, 130 mg or 200 mg.

20. (original) The method of claim 16, wherein the administering comprises intramuscular injection or intravenous injection.

21. (previously presented) The method as in claim 2, wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.

22. (currently amended) A pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium of claim 1 [[2]].

23. (currently amended) A method of treating an individual in need of Phenobarbital Sodium, the method comprising:

adding saline or dextrose to a composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium immediately prior to administration to form a Phenobarbital Sodium solution with no more than 0.2% phenylethylacetylurea (PEAU), the lyophilized Phenobarbital Sodium of claim 1 [[2]]; and

administering the Phenobarbital Sodium solution to the individual.

REMARKS/ARGUMENTS

The Office action dated 26 February 2021 has been received and carefully considered. By this amendment, claims 1-16, 22-23 are amended. After entry of this Amendment, claims 1-23 will be pending. With these amendments, no new matter is added. In view of these amendments and the following remarks, Applicants respectfully request reconsideration.

Claim Amendments

Independent claims 1, 2, and 16 as well as their respective dependent claims 3, 8-12, 14, 15, 22, 23 have been amended to clarify that lyophilized amorphous Phenobarbital Sodium is provided in a storage-stable powder form. This is disclosed at least in para [0013], [0020] and many others. No new matter has been added.

35 U.S.C. §102

The Office maintains the rejection of **claim 1** under 35 U.S.C. §102(b) as being anticipated by Sun et al (US 20100035904). In view of the amendments herein, and arguments below, Applicant respectfully disagrees.

As amended, claim 1 requires the limitations of the composition "*comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution*". These limitations are not taught or suggested by the cited reference.

The Office maintains it rejection of claims 1 and 22 suggesting that Sun discloses the same chemical composition of the Phenobarbital Sodium, as well as amorphous forms of phenobarbital salts, which would necessarily anticipate desired storage-stable properties of the composition of the present invention. As to new limitations of providing the composition in lyophilized form that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, the Office argues that "..."new limitations" are considered inherent in the properties and are necessarily present unless there is evidence showing that this, in fact, is not the case."

Regarding the Office's arguments, it must be appreciated that applicant does not claim the compound phenobarbital *per se* as a molecular entity and its inherent properties, but a specific

morphological form of Phenobarbital Sodium. As established at Paragraph 5 in the attached Declaration, the chemical structure of a compound *per se* does not define by itself the entire range of attributes associated with the compound, and in particular the storage stability of specific morphological forms of Phenobarbital Sodium. Physical characteristics, such as a dry *vs* liquid form during storage, play significant role in the process of Phenobarbital Sodium degradation, with the presence of PEAU being one of the measurable consequences of such degradation. Examples of an unexpectedly superior storage stability of the lyophilized amorphous powder form of Phenobarbital Sodium as compared to a liquid solution of the same compound are discussed through the specification, for example in tables 2 and 3.

Attention is drawn to the fact that Sun broadly mentions the term "amorphous" with general reference to phenobarbital salts but fails to teach lyophilized amorphous forms of Phenobarbital Sodium. In addition, Sun does not teach or even recognize a connection between lyophilized amorphous forms of these salts and any storage stability, let along long-term stability of such product. As is stated at Paragraph 6 of the Declaration, the person skilled in the art would not associate lyophilized amorphous forms of Phenobarbital Sodium with long-term storage stability. As such, the claimed composition provided as a lyophilized amorphous powder form would not be normally expected to demonstrate superior storage stability as was unexpectedly discovered by the inventors of the present patent application. See also Paragraph 7 of the Declaration.

Moreover, as already stated in the previous response, the term "*lyophilized*" in conjunction with Phenobarbital Sodium is not merely reflective of a general process that would necessarily produce the same product as apparently alleged by the Office. To that end, it is pointed out that the claims expressly recite a lyophilized *amorphous* product having *specific stability criteria*: a storage-stable high-purity form that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution. Paragraph 8 in the Declaration reiterates the point that not every lyophilization process of a compound will necessarily result in the same product having same physicochemical properties.

Therefore, Sun does not inherently anticipate superior storage stability. Thus, for at least these reasons, the cited reference fails to teach or suggest each and every limitation of claim 1, and Applicant respectfully requests the above rejection be withdrawn.

35 U.S.C. §103

The Office rejected **claims 2, 6, 8-13, 15, and 21-22** under 35 U.S.C. §103 as obvious over Sun in view of Parker and Snyder. In view of the amendments herein, and arguments below, Applicant respectfully disagrees.

As amended, claim 2 requires a step of "*lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium.*" Storing the Phenobarbital Sodium in a lyophilized amorphous dry powder form produced by the steps of claim 2 are not taught, suggested, or motivated by the cited reference, alone or in combination with other references.

As discussed in greater detail in the previous response, Parker describes a liquid solution form of Phenobarbital Sodium with pH of between 9 and 12. The present application and the attached Declaration provide factual support that storage of a liquid solution of Phenobarbital Sodium, even with the same pH as used in the process of producing a lyophilized amorphous dry powder form thereof, does not lead to a superior storage stability. In fact, storage of the liquid form of Phenobarbital Sodium is shown to produce a high level of PEAU, up to 5.8% in the test results described in Table 2 of the specification. See Paragraph 9 of the Declaration.

Parker explains one mechanism of degradation of Phenobarbital Sodium in the presence of water in paragraph [0004], namely "the presence of the hydroxyl ions in the formulation from the water results in a hydrolysis pathway that can destroy the phenobarbital ring complex." Parker goes on to say that "this destruction results in the possible formation of impurities including harmful degradants or precipitates." Parker also recognizes later in paragraph [0004] that "it is desired to minimize or preferably eliminate any impurities in commercial phenobarbital sodium injection formulations."

Parker further states in paragraph [0005] "The solution to the problem then easily becomes removal of water from the formulation. This is easier said than done ..." Parker cannot offer a suitable way to remove water from the formulation and instead provides a compound that includes not only Phenobarbital Sodium but also other ingredients such as C1-C4 alcohol and glycol, which when mixed together with Phenobarbital Sodium under certain conditions provide a desired stable

storage behavior. Therefore, Parker teaches away from the present invention, which does not rely on other additives to achieve extended storage stability but rather produces a particular form of a dry powder of Phenobarbital Sodium using a described process to achieve a desired result. Indeed, it should also be appreciated that the method of claim 2 and dependent claims relies on a fairly alkaline pH in the lyophilization solution, which is a factor that Parker considers detrimental to stability as noted by Parker in [0004].

Once more it is noted that Parker is concerned with storage stability of a liquid composition. However, such composition is not relevant to the claims as there is no known nexus between the storage stability of a liquid formulation at a certain pH and a lyophilized amorphous form of Phenobarbital Sodium. See Paragraph 10 of the Declaration.

Still further, with regard to Parker, it must be appreciated that Parker's formulation is a liquid formulation that contains ethanol, propylene glycol, and because the pH is adjusted with HCl, the liquid formulation shows higher levels of impurities. This is due to the fact that HCl is aqueous (note that HCl is a gas at standard conditions, and that commercially available HCl for pH adjustments are always aqueous solutions of hydrochloric acid) and therefore contributes to water content. In contrast, the claimed formulation avoids pH adjustment with HCl (in fact is basic) and uses optimized lyophilization conditions that resulted in a finished product that has substantially reduced residual moisture content, which increases the shelf life of the finished product. Indeed, in spite of being amorphous, the moisture content over a period of 15 months does not significantly increase and as such the final finished product will not suffer from an increase of impurities.

With respect to the combination of Parker with Sun, applicant notes that the Office failed to offer a well-reasoned rebuttal to the argument advanced in the previous response that Parker teaches a certain pH in the solution during the storage phase and not the solution used as a starting point for the lyophilization process. It is not clear to the applicant what the Office means by stating on page 9 that the rejection is based on combination of references. Lyophilization is not mentioned anywhere in Parker and there is no teaching to use the Phenobarbital Sodium solution alone, without alcohol and glycol additives. Indeed, Parker is concerned with storage stability in liquid form and as such fails to provide any motivation or reasonable expectation of success that

lyophilization would result in a stable product, let alone a powder form of lyophilized amorphous Phenobarbital sodium with the stability parameters as presently claimed.

The Office relies on Snyder to disclose lyophilization as a way to remove water and improve storage stability of a Phenobarbital Sodium composition. As stated at Paragraph 8 in the Declaration, a mere generic discussion of lyophilization does not provide sufficient teaching or motivation to a skilled artisan in this field to produce the lyophilized amorphous powder form of Phenobarbital Sodium as described in the specification and presently claimed. It is once more emphasized that not all lyophilization processes are the same and as such will produce products with significantly different product parameters. The Applicant unexpectedly discovered that the lyophilization process as described will produce a storage stable lyophilized amorphous powder form of the Phenobarbital Sodium that is characterized by forming no more that 0.2% PEAU when reconstituted in an aqueous solution. Snyder is devoid of such teaching or even hint in that direction.

As discussed at great length in the previous response, Snyder is also silent as to the desired pH range of the drug solution prior to the initiation of the lyophilization process, or about any other water-related parameters as described in depth in the specification, for example providing the water for adding the Phenobarbital Sodium in a predetermined temperature range as specified in claim 6.

Claim 3 is presently amended to describe a product (lyophilized amorphous dry powder of Phenobarbital Sodium) and the process of making thereof by a specific lyophilization process in which an aqueous Phenobarbital Sodium solution with a pH at or between 9.2 to 10.2 is formed, and in which that solution is then subjected to lyophilization. Using this narrowly defined initial solution as a starting point for the lyophilization process has not been described or suggested in the art of record.

Claims 4-15 provide additional unique details of the ingredients for and specific conditions of the lyophilization process that are not fairly described or suggested in the prior art.

Claims 16-20 are rejected as obvious over Kelly and Sun. The Office again relies on the argument that the storage stable properties of the Phenobarbital Sodium powder as described in

the specification are inherently disclosed by Sun describing the same chemical composition as the present invention. For reasons identified above with regard to claims 1 and 2, the Applicant respectfully disagrees.

Claim 21 describes further specifics of the lyophilization process, namely "*the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.*" Although the Office lists claim 21 as rejected under 35 USC 103 as obvious over Sun in view of Parker and Snyder, no specific reasons are provided for this rejection. None of the prior art references described lyophilization process details described in claim 21.

Finally, a presently amended claim 23 describes the use of the lyophilized amorphous powder form of Phenobarbital Sodium made according to the methods of the present invention described in claim 2 for treating an individual in need of Phenobarbital Sodium. The Office did not address this independent claim at all in the Final Office Action.

Request For Allowance

Claims 1-23 are pending in this application. Applicant requests allowance of all pending claims.

Respectfully submitted,
Umberg Zipser LLP

Date: May 13, 2021

By: /Martin Fessenmaier Ph.D./
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

Mail Stop Amendments
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, the undersigned, Dr. Dasaradhi Lakakraju, hereby declare as follows:

1. I have obtained my PhD degree in the field of Synthetic Organic Chemistry in 1989 from Osmania University in India, and have worked as a Postdoctoral fellow in the Department of Chemistry at University of Durham in England, New Mexico State University, New Mexico, and at the Department of Pharmacology in State University of New York, at Stony Brook, New York. I have over 24 years of analytical chemistry experience in the pharmaceutical industry.

2. I have published 24 papers in peer reviewed Journals and patents in formulation development. I am presently the Chief Scientific Officer at Nivagen Pharmaceuticals Inc. I am an inventor in the above referenced application.

3. In connection with this declaration, I have reviewed the following materials in detail, bringing to bear my knowledge of one who is skilled in the relevant art of all aspects of the

pharmaceutical chemistry and galenics, and especially in the field of drug formulation using lyophilization.

3.1. US Patent Application No. 17/025,881

3.2. Non-final Office Action dated 30 November 2020

3.3. Response to the Non-final Office Action dated 12 February 2021

3.4. Final Office Action dated 26 February 2021

3.5. US Patent Application Publication No. US2010/0035904

3.6. US Patent Application Publication No. US2017/0143719

3.7. US Patent No. 6.077.545

3.8. Snyder Meg. Lyophilization: The Basics. Reference cited by the USPTO, saved on 11/19/2020 from www.drugdiscoverytrends.com

3.9. Kelly L, et al. Phenobarbital Sodium. Reference cited by the USPTO, saved on 11/18/2020 from www.lhsc.on.ca

4. With respect to the above referenced examination, I hereby declare the following:

5. The Examiner stated on page 3 of the office action that "*if the prior art teaches the identical chemical structure and the same lyophilized amorphous [form], the properties e.g. storage-stable and the amount of phenyl ethyl acetylurea (PEAU) when reconstituted in an aqueous solution applicant claims are necessarily present.*" Such argument is incorrect and inconsistent with the understanding of a skilled artisan in the field of lyophilization. Chemical structure alone does not fully define storage stability and longevity of Phenobarbital Sodium. Indeed, both the physical form of the composition as well as the chemical structure define the storage stability of many compounds, and certainly that of Phenobarbital Sodium.

6. The skilled artisan would not associate or predict long-term storage stability of Phenobarbital Sodium with a lyophilized amorphous form of Phenobarbital Sodium. Phenobarbital Sodium in a liquid solution form behaves very differently during storage as compared to the Phenobarbital Sodium in a lyophilized amorphous powder form. A liquid solution form allows the chemical processes of degradation of Phenobarbital Sodium and formation of PEAU to continue throughout the storage duration, which is not the case with the dry powder form of the composition. To wit, examples of a superior storage stability of the powder form are presented in numerous places in the application, for example in Tables 2 and 3. These tables show a strong presence of Phenobarbital Sodium degradation products, namely PEAU, at 3 months and at 6 months in the liquid solution measured anywhere between 0.9 and 5.8% as compared with consistent lack of PEAU (below quantification limit) for a powder form of the compound. Lack of PEAU continued to persist even for as long as 15 months of storage, further highlighting a superiority of storage stability of the powder form over a conventional liquid solution form of Phenobarbital Sodium.

7. While crystalline forms of Phenobarbital salts are known to be stable during storage, amorphous forms of these salts are not conventionally understood as storage-stable. A skilled artisan in the art would appreciate that the amorphous form of Phenobarbital Sodium lacks ordered crystalline structure and tight packing, which would normally prevent or substantially reduce degradation processes. Amorphous forms do not provide such limitations and therefore would be normally considered as inferior to crystalline forms. Because of that general notion, the storage performance of the lyophilized amorphous form of Phenobarbital Sodium is surprising and unexpected.

8. Lyophilization is a general term for water removal from a composition via sublimation, and numerous process parameters such as temperatures, temperature gradients, degree of vacuum applied, and/or time periods at specific temperatures and partial vacuum can significantly affect the physicochemical properties of the lyophilized product. It is well known to the skilled artisan that not every lyophilization process of a compound will necessarily result in the same product having same physicochemical properties. Optimization of lyophilization technique to suit the formulation of interest to obtain a stable

finished product is not described in the literature and is very critical to obtain a stable finished product.

9. The Examiner stated on page 5 of the Final Office Action that "Parker teaches that stable phenobarbital sodium solutions for injection include phenobarbital sodium ... with a pH of between 9 and 12." Later in the same paragraph the Examiner stated that "Parker teaches that the solutions for injection do not generate impurities including phenyl ethyl acetyl urea (PEAU) during extended storage." This statement is incorrect as the skilled artisan in the field of stable storage forms of various liquid and dry compounds would understand that liquid form of Phenobarbital Sodium is inherently prone to degradation and forms PEAU over time. The present application contains multiple experimental examples of detected impurities in the liquid form of Phenobarbital Sodium at similar pH ranges as it was stored over a few months. For example, Table 2 in the application shows test results of a liquid solution of Phenobarbital Sodium at the pH in the range of 9.3 to 9.6 stored over 6 months at 25 and 40 degrees C and various relative humidity. PEAU content is shown to increase steadily over the 6-month test period reaching as much as 5.8% (see *e.g.*, *Journal of Neurology, Neurosurgery, and Psychiatry* 1982;45:1113-1118; and *British Pharmacological Society, 3rd-5th January 615P*: Pheneturide, a more potent liver enzyme inducer in man than phenobarbitone? A. N. LATHAM and A. RICHENS). At the same time, a lyophilized powder form of Phenobarbital Sodium as prepared according to the methods described in the application has shown a much superior storage stability and PEAU levels below quantitation limits (BQL) – even when the test was extended to as long as 15 months.

10. With respect to storage stability of Phenobarbital Sodium, there is no connection between the pH of a liquid form of Phenobarbital Sodium and a lyophilized amorphous powder form. The pH of the liquid solution during storage is not indicative of the storage stability characteristics of a dry powder form of Phenobarbital Sodium.

Executed at Davis, CA, this 10th day of May, 2021.

By: _____
(Dr. Dasaradhi Lakakraju, Chief Scientific Officer)

Electronic Patent Application Fee Transmittal				
Application Number:	17025881			
Filing Date:	18-Sep-2020			
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt			
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu			
Filer:	Martin Fessenmaier/Danielle Moroz			
Attorney Docket Number:	104078.0001US			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
RCE- 1ST REQUEST	2801	1	680	680
		Total in USD ($)		**680**

To: rdean@umbergzipser.com,patents@umbergzipser.com,
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Jun 01, 2021 04:23:07 AM

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Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614
UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
17025881	CTNF	06/01/2021	104078.0001US
	892	06/01/2021	104078.0001US

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US	8113

149344 7590 06/01/2021

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

EXAMINER	
ZHANG, YANZHI	

ART UNIT	PAPER NUMBER
1617	

NOTIFICATION DATE	DELIVERY MODE
06/01/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@umbergzipser.com
rdean@umbergzipser.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 17/025,881	Applicant(s) Chodavarapu et al.	
	Examiner YANZHI ZHANG	Art Unit 1617	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 05/13/21.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-23 is/are pending in the application.

5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☑ Claim(s) 1-23 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on ____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. ____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date ____.

4) ☐ Other: ____.

Notice of Pre-AIA or AIA Status

The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Claim Status

This action is a response to papers filed on May 13, 2021. Claims 1-16, and 22-23 have been amended. No claim has been newly added or canceled. Accordingly, claims 1-23 are pending in the application and under consideration on the merit.

Claim Objection

Claim 22 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 1. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 608.01(m).

Claim 22 is not addressed individually since the prior teaches the limitations of claims 1 and 2 would meet the requirement of claim 22.

Claim Rejections - 35 U.S.C. 103

The text of those sections of Title 35 of the U.S. Code not included in this action can be found in a prior Office action.

Claims 1-3, 8-13, 15, and 21-22 are rejected under 35 U.S.C. 103 as obvious over Sun et al ("Sun", US 20100035904 A1, publication date: February 11, 2010) in view of Parker et

al ("Parker", US 20170143719 A1, publication date: May 25, 2017) and Snyder (non-patent literature, Drug discovery and development, pp. 1-5, March 7, 2017).

Claims 1-3, 8-13, 15, and 21-22 embrace a composition and method of producing a storage-stable powder form of lyophilized Phenobarbital sodium that forms no more than 0.2% phenyl ethyl acetyl urea (PEAU) when reconstituted in an aqueous solution, the method comprising: adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution; if necessary, adjusting the pH of the phenobarbital sodium solution to or between 9.2 to 10.2; and lyophilizing the Phenobarbital Sodium solution and a method of using thereof.

In addition, claims 1, 2 (method claim), and 22 use the open-ended transitional phrase "comprising". Thus, they allow for the presence of additional unrecited steps or components.

Sun is directed to **phenobarbital** salts; **methods of making**; and methods of use thereof (title). Sun teaches non-crystalline or **amorphous forms** of phenobarbital salts including **phenobarbital sodium** ([0011], the same drug in the instant claims 1-2). Sun also teaches that preparation of phenobarbital sodium salt is achieved by dissolving phenobarbital and sodium hydroxide in acetone followed by removal of the acetone ([0055]). Sun further teaches that the concentration of phenobarbital in selected buffers or **pH conditions** will be determined using a **stability indicating assay** that distinguishes the active agent from its degradation products, and also detects any degradation that may appear in the salt solution ([0060], read on the limitation of the instant claim 12). Sun recognizes that phenobarbital sodium is a known salt freely soluble in water in contrast to the free acid form, but phenobarbital sodium is hygroscopic and degrades when exposed to humid environments ([0004]).

While teaching preparation of amorphous phenobarbital sodium by adjusting pH conditions and solvent removal, Sun does not expressly teach the specific pH range as claimed

nor aqueous solvent (water) by lyophilization. These deficiencies are cured by Parker and Snyder, respectively.

Parker is directed to **stable formulation** of phenobarbital sodium injection (title, read on the limitation of the instant claim 22). Parker teaches that stable phenobarbital sodium **solutions for injection** include phenobarbital sodium in an amount of 15 to 200 mg/mL; a C1-C4 alcohol in an amount of 105 to 160 mg/mL; and propylene glycol in an amount of between 620 and 830 mg/mL and have a pH of between 9 and 12 (abstract, read on the limitations of aq. solution in the instant claims 1 and 2). Parker also teaches that these solutions, preferably, have a **pH from 9.2 to 10.2**, with the pH of the solution adjusted to that range by adding a sufficient amount of acid or base ([0034], read on the limitations of pH range in the instant claims 2 and 2). Parker further teaches that the solutions for injection are extremely stable and do not generate any significant amounts of impurities, including phenyl ethyl acetyl urea (PEAU) and phenyl butyric acid (PBA, during extended storage ([0017]), i.e. **impurity-free** formulations([0016]), implying the limitation of no more than 0.2%, o.1% or 0.05% PEAU in the instant claims 1-2, 8-9, 12, and 23).

Snyder is directed to Lyophilization: the Basics (title). Snyder teaches that **lyophilization** is defined as a freeze-drying process to **remove water from a product** after it is frozen and placed under a vacuum (the 1st paragraph on page 1/5, implying the solvent of water). Snyder indicates that if the bulk drug ingredients are not stable in liquid or frozen form due to chemical reactions, degradation, aggregation, biological growth, heat sensitivity, lyophilization is necessary (the 3rd paragraph on page 1/5). Snyder teaches that lyophilization enables longer shelf life (enhancing stability), makes it much easier to process the product including removing water without having to heat the product excessively and reconstituting the product rapidly and

easily (the 1st paragraph on page 2/5). Snyder also teaches that the lyophilization process involves the following steps (the last paragraph on page 3/5):

1. Fill tray with liquid solution (implying the 1st step of adding a drug to water to form the liquid solution in the instant claim 1),

2. Carry trays over to dryer and load

3. Lyophilization (including those three stages)

4. Remove tray from dryer and remove powder from try into another bag or sealed **container** (interpreted as a vial, read on the limitation in the instant claim 15). Snyder further teaches that some of the **advantages of lyophilization**, according to the FDA, include:

- Processing a liquid with ease (and thereby simplifying aseptic handling)
- Enhancing the stability of a **dry powder** as well as the product stability in a dry state
- Removing water without having to heat the product excessively
- Dissolution of reconstituted product (rapidly and easily).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose lyophilization as taught by Snyder as the particular means for an aqueous drug solution because the particular option identified by Snyder are predictable solutions to the problem of drying an unstable drug like phenobarbital sodium, for example, enhancing the stability of a dry powder as well as the product stability in a dry state. The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing the option. See MPEP 2141, part (III)(A) and (D). Additionally, one of ordinary skill in the art would take advantages of lyophilization taught by Snyder as set forth above.

Regarding the limitation of "a storage-stable powder form of" lyophilized amorphous in claims 1 and 2, it is reasonable to believe that, when lyophilized as taught by Snyder, the

pharmaceutical composition of Phenobarbital Sodium has the same physical properties as claimed.

Regarding the preamble of "no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution" in claim 2, "no more than 0.1% phenylethylacetylurea" in claim 8, "no more than 0.05% phenylethylacetylurea" in claim 9, no detectable amount of 2-ethyl-2-phenylmalonamide, etc. in claim 10, and stable up to at least 6 months at accelerated stability conditions in claim 11; these are considered properties of the lyophilized Phenobarbital sodium. The lyophilized Phenobarbital sodium as set forth in the rejection above would meet these limitation. In the absence of the USPTO to have at its disposal the tools or facilities deemed necessary to make physical determinations of this sort, Applicants have the burden to show that this, in fact, is not the case.

Claims 4-7, and 14 are rejected under 35 U.S.C. 103 as obvious over Sun et al ("Sun", US 20100035904 A1, publication date: February 11, 2010) in view of Parker et al ("Parker", US 20170143719 A1, publication date: May 25, 2017) and Snyder (non-patent literature, Drug discovery and development, pp. 1-5, March 7, 2017) as applied to claims 1-3, 8-13, 15, and 21-22 in further view of Roskos et al ("Roskos", US 6077545 A, date issued: June 20, 2000) and Dietz et al (non-patent literature, Pharmaceutical Research, vol. 5, No. 12, pp. 1-5, 1988).

The reference of Dietz et al is provided by applicant in the form of IDS dated 11/30/20.

The teaching of Sun, Parker, and Snyder have been discussed as applied to claims 1-3, 8-13, 15, and 21-22. Sun, Parker, and Snyder do not expressly teach the water is water for injection (WFI), nor nitrogen purge. These deficiencies are cured by Roskos.

Roskos is directed to an improved diluent for the preparation of cisplatin suspensions from a lyophilized powder (abstract). Roskos teaches that lyophilized CDDP (a drug for cancer) is intended for reconstitution into a solution and the standard vehicle for such solution reconstitution is **Water for Injection** (WFI) (col. 3, lines 4-6, read on the limitation of the instant claim 4). Roskos teaches that the solution of example 1 is sparged with nitrogen for 30 minutes (gas flushing), when the required amount of epinephrine is added, and the mixture is stirred under a nitrogen blanket until the epinephrine dissolved, **pH is adjusted** with HCl and/or NaOH (Table of col. 5) if necessary, and additional WFI was added to achieve the final desired volume. The solution was **sparged with nitrogen** for an additional 30 minutes, and then sterile **filtered** through a 0.22 micron filter. Filtered solution was filled into 5 ml vials, the vial headspace was flushed with nitrogen, and the vials are stoppered (interpreted as sealed) (col. 5, lines 45-58, read on the limitations of the instant claims 4-5, 7, and 14).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose nitrogen sparge and filtering prior to adding the drug as taught by Roskos as the particular means to ensure the drug is stable and meet the Food and drug administration requirements. The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing the steps because Roskos provides the exact solution of processing a stable lyophilized drug product. See MPEP 2141, part (III)(A) and (G). In addition, selection of any order of performing process steps is prima facie obvious in the absence of unexpected results (see MPEP 2144).

Regarding the water is cooled to or between 2 to 8°C prior to adding the Phenobarbital Sodium in claim 6, Dietz teaches that heat facilitating degradation of Phenobarbital Sodium. Dietz teaches that the aqueous solution of phenobarbital sodium degrades fastest (see a heat facilitated

degradation profile in Fig. 1 on page 804 and the average percentage in Table 1 on page 805). Thus, it would have been obvious to cool the water to avoid or slow down the degradation.

Claim Rejections - 35 U.S.C. 103

The text of those sections of Title 35 of the U.S. Code not included in this action can be found in a prior Office action.

Claims 16-20 are rejected under 35 U.S.C. 103 as obvious over Kelly et al ("Kelly", non-patent literature, Critical Care Trauma Centre, pp. 1-3, last update: October 11, 2018) and Sun et al ("Sun", US 20100035904 A1, publication-date: February 11, 2010).

Claims 16-20 embrace a method of treating an individual in need of Phenobarbital Sodium, the method comprising: adding saline or dextrose to a composition comprising lyophilized amorphous Phenobarbital Sodium immediately prior to administration to form a Phenobarbital Sodium solution; and administering the Phenobarbital Sodium solution to the individual.

Kelly is directed to **phenobarbital sodium** for **intravenous** (IV) direct injection or infusion (title, and the section of Administration on page 1, read on the limitation of the drug in the instant claim 16 and the administering routes in the instant claim 20). Kelly discloses that the drug, anticonvulsant, sedative, is diluted to 10mL with sodium chloride 0.9%, dextrose 5% or Ringer's Lactate and is used to treat seizures (i.e. epilepsy) (the section of Classification on page 1, read on the limitations of the instant claims 16-17). Kelly also discloses the loading dose is 10 - 15 mg/kg IV or 1000 mg in 250 mL for infusion (the section of Dose and Administration on page 1, read on the limitation of the instant claims 19 and 23).

While teaching phenobarbital sodium for intravenous Kelly does not expressly teach phenobarbital sodium is a lyophilized amorphous product as claimed. This deficiency is cured by Sun.

Sun is directed to phenobarbital salts; methods of making; and methods of use thereof (title). Sun discloses that **phenobarbital sodium** is a known salt freely soluble in water in contrast to the free acid form ([0004], the same drug in the instant claim 1). Sun also discloses non-crystalline or **amorphous forms** of phenobarbital salts including phenobarbital sodium ([0011], the same physical form in the instant claim 16).

It would have been obvious for one of ordinary skill in the art, as of the effective filing date of the claimed invention, to choose amorphous form of phenobarbital sodium as taught by Sun as the particular drug to be incorporated into the method of Kelly because all of the particular options identified by Sun are predictable solutions to the drug formulations,. The person of ordinary skill in the art would have a reasonable expectation to be of success in choosing any of those options. See MPEP 2143, part (I)(B) or (E).

Response to Arguments and ***Declaration***

Applicant's Declaration under 37 CFR 1.132 filed 05/13/2021 by Dr. Dasaradhi Lakakraju and arguments pertaining to the obviousness of the instant claims are not persuasive for the following reasons.

Applicant argues (p. ¾ of declaration) that the skilled artisan would not associate or predict long-term storage stability of Phenobarbital Sodium with a lyophilized amorphous form of Phenobarbital Sodium. Phenobarbital Sodium in a liquid solution form behaves very differently during storage as compared to the Phenobarbital Sodium in a lyophilized amorphous powder form.

A liquid solution form allows the chemical processes of degradation of Phenobarbital Sodium and formation of PEAU to continue throughout the storage duration, which is not the ease with the dry powder form of the composition.

In response, applicant is reminded that the claimed subject matter is a pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium and method of producing thereof. It is agreed that Phenobarbital Sodium in a liquid solution form behaves very differently during storage as compared to the Phenobarbital Sodium in a lyophilized amorphous powder form. In addition, crystalline form is not the claimed subject matter. Thus, the property of crystalline form is irrelevant.

Second, applicant argues that lyophilization is a general term tor water removal from a composition via sublimation, and numerous process parameters such as temperatures, temperature gradients, degree of vacuum applied, and/or time periods at specific temperatures and partial vacuum can significantly affect the physicochemical properties of the lyophilized product.

In response, the claimed pharmaceutical composition and the methods of making thereof do NOT have any parameters, except pH which was taught by Parker.

Applicant also argues that Table 2 in the application shows test results of a liquid solution of Phenobarbital Sodium at the pH in the range of 9.3 to 9.6 stored over 6 months at 25 and 40 degree.

In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., pH in the range of 9.3 to 9.6) are not recited in the rejected claims. The claimed pH range is broader than 9.3 to 9.6. Although the claims are interpreted in light of the specification, limitations from the

specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993).

Relevant Art

Nahata et al (non-patent literature, titled Stability of phenobarbital sodium diluted in 0.9% sodium chloride injection; Am J Hosp Pharm, 1986 Feb; vol. 43(2), pp.384-5; full article) is provided, but not cited, to show the state of art at the time when the invention was filed.

CONCLUSION

No claim is allowed.

CONTACT INFORMATION

Any inquiry concerning this communication or earlier communications from the examiner should be directed to YANZHI ZHANG whose telephone number is (571)272-3117. The examiner can normally be reached on Monday-Friday 8am-5pm.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Johann Richter can be reached on 5712720646. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/YANZHI ZHANG/
Primary Examiner, Art Unit 1617

Notice of References Cited	Application/Control No. 17/025,881	Applicant(s)/Patent Under Reexamination Chodavarapu et al.	
	Examiner YANZHI ZHANG	Art Unit 1617	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
	A					
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	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Nahata et al, title: Stability of phenobarbital sodium diluted in 0.9% sodium chloride injection; Am J Hosp Pharm, 1986 Feb; vol. 43(2), pp.384-5; full article) (Year: 1986)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.



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The **pH** range is 9.2-10.2. The **sodium** salt of **phenobarbital** occurs as a white, slightly bitter powder, crystalline granules or flaky crystals, it is soluble in alcohol and practically insoluble in ether or chloroform.

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The stability of solutions of phenobarbital sodium

A 10% solution of phenobarbital sodium saturated with the free acid has a pH of ... solution of phenobarbital suitable for intramuscular injection can be made by ...

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WO2017085687A1 - Stable formulation of phenobarbital ...

Stable phenobarbital sodium solutions for injection that do not generate ... mg/mL and have a pH of between 9 and 12 which is adjusted to that range by adding ...

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PHENOBARBITONE/PHENOBARBITONE SODIUM

Phenobarbitone sodium is very soluble in water (1 in 3) and soluble in alcohol (1 in 25) ... precipitated from solutions of phenobarbitone sodium depending on the pH, ... Phenobarbitone sodium injection is usually formulated with ethanol and ...

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US Patent for Stable formulation of phenobarbital sodium ...

Feb 27, 2018 – Stable phenobarbital sodium solutions for injection that do not ... and antioxidants in order to form stable solutions at pH values of 5 to 8.

Searches related to pH of sodium phenobarbital injection solution

phenobarbital sodium injection	phenobarbital **dilution in normal saline**
phenobarbital **intramuscular** injection	**phenobarbitone** injection **1mg**
phenobarbital **iv dilution**	phenobarbital injection **200mg 2ml**
phenobarbital solution	phenobarbital **iv push dilution**

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Stability of phenobarbital sodium diluted in 0.9% sodium chloride injection

MILAP C. NAHATA, THOMAS F. HIPPLE, AND STEVEN D. STRAUSBAUGH

Abstract: The stability of phenobarbital sodium diluted to 10 mg/mL in 0.9% sodium chloride injection was studied at an unadjusted pH (8.5) and an adjusted pH (10.0) at 4 °C over a four-week period.

The phenobarbital concentrations were measured before dilution and at 1, 2, 3, 7, 14, and 28 days using high-performance liquid chromatography.

The observed phenobarbital concentrations ranged from 98.0 to 106.0% of initial concentration at the unadjusted pH and from 96.7 to 108.5% of initial concentration at the adjusted pH.

The diluted phenobarbital sodium was stable over a four-week period at 4 °C without the need for pH adjustment.

Index terms: Additives; Anxiolytics, sedatives and hypnotics; Concentration; Diluents; Dilutions; Hydrogen ion concentration; Incompatibilities; Injections; Phenobarbital sodium; Sodium chloride; Stability

Am J Hosp Pharm. 1986; 43:384–5

Seizure disorders occur frequently in newborn infants. It has been reported that 20% of infants below 2500 g birth weight may have seizures.[1] Phenobarbital is the most commonly used drug for the treatment of seizures in these patients. Each milliliter of the commercially available injectable formulations includes phenobarbital sodium 65 mg and 0.678 mL of propylene glycol. There are two potential problems with the use of this product in newborn infants. First, because the maintenance dose of phenobarbital sodium is 3–5 mg per kilogram of body weight, it may be difficult to measure a dosage volume as low as 0.05 mL accurately to provide 3.25 mg of phenobarbital for a patient weighing about 1000 g. Second, serum propylene glycol concentrations of 70 mg/dL, which may cause hyperosmolality in small infants, have been attributed to the use of phenobarbital sodium formulations containing propylene glycol.[2]

One approach to minimize errors in the measurement of small volumes would be to dilute the phenobarbital product before dispensing. The dilution with its base, propylene glycol, which has been added to increase the stability of phenobarbital, was not considered because of its potential adverse effects. The product can be diluted with 0.9% sodium chloride injection but it is important to ensure the stability of phenobarbital sodium in the diluted product. In a preliminary observation, we found that the pH of this diluted phenobarbital sodium was below the USP recommendations (9.2–10.2).[3]

The objective of this study was to determine the stability of phenobarbital sodium in 0.9% sodium chloride injection at an unadjusted and adjusted pH at 4 °C.

Methods

Phenobarbital sodium (Elkins Sinn, Inc., Cherry Hill, NJ, lot 074160) injection was used for the study. One milliliter of this product containing phenobarbital sodium 65 mg was diluted with 5.5 mL of 0.9% sodium chloride injection to yield the final phenobarbital concentration of 10 mg/mL in glass vials. The pH of this diluted product was found to be 8.5. To bring the pH of the diluted product within the pH range recommended in the USP, the pH was adjusted to 10 using 0.1-*N* sodium hydroxide. The pH was measured using a digital pH meter.

Initial phenobarbital concentrations were measured within two hours of dilution at the unadjusted and adjusted pH values before storage in the refrigerator at 4 °C. Phenobarbital concentrations were also measured on days 1, 2, 3, 7, 14, and 28 to determine the stability of the diluted phenobarbital. Phenobarbital was measured in duplicate samples of two replicate solutions using a commercially available high-performance liquid chromatography (HPLC) method (Laboratory Data Control, Riviera Beach, FL 33404). The samples were diluted to a phenobarbital concentration of 1 mg/mL just before the measurement of phenobarbital concentration by HPLC. The HPLC method uses a reverse-phase partition chromatography and Spherisorb S5 ODS column (220 × 4 mm) at the following conditions: mobile phase, 50% methanol in water; wavelength, 235 nm; and flow rate, 1 mL/min. The coefficient of variation of the assay at the phenobarbital concentration of 1 mg/mL was less than 4%. The assay was considered stability indicating, based on the decomposition of phenobarbital and lack of

MILAP C. NAHATA, PHARM.D., is Associate Professor of Pharmacy and Pediatrics, Colleges of Pharmacy and Medicine, The Ohio State University (OSU) and Children's Hospital. THOMAS F. HIPPLE is Director of Pharmacy, Children's Hospital. STEVEN D. STRAUSBAUGH is a pharmacy junior, College of Pharmacy, OSU, Columbus.

Address reprint requests to Dr. Nahata at the College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210.

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interference of an unknown peak with phenobarbital peak after boiling at 100 °C for 16 hours (Figure 1). The Wilcoxon nonparametric statistical test was used to determine the significance of change in phenobarbital concentrations during storage at the two pH values.

Results

The concentrations of phenobarbital sodium diluted in 0.9% sodium chloride injection during storage over a four-week period in the refrigerator are reported in Table 1. The phenobarbital concentrations ranged from 98.0 to 106.0% of initial concentration at the unadjusted pH of 8.5 and from 96.7 to 108.5% of initial concentration at the adjusted pH of 10.0. The changes in phenobarbital concentration during the storage period were not significant. Thus, the diluted phenobarbital was stable in 0.9% sodium chloride injection without the need for pH adjustment during a four-week period at 4 °C.

Figure 1. Chromatograms of phenobarbital (PB) at room temperature (A) and after boiling at 100 °C for 16 hours (B); an unknown peak (U) occurred after boiling.

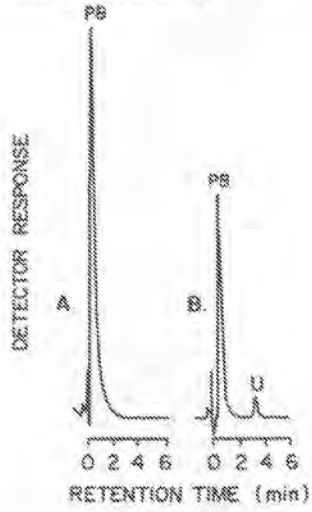


Table 1.
Stability of Phenobarbital Sodium Diluted in 0.9% Sodium Chloride Injection at Two pH Values at 4 °C

Days after Dilution	Percent of Initial Phenobarbital Sodium Concentration at 4 °C	
	Unadjusted pH = 8.5	Adjusted pH = 10.0
1	100.0	101.0
2	101.4	103.4
3	98.1	96.7
7	106.0	105.4
14	101.5	108.5
28	98.0	102.9

Discussion

Our data suggest that phenobarbital sodium can be diluted from the commercially available concentration of 65 mg/mL to a concentration of 10 mg/mL in 0.9% sodium chloride injection for use in infants. The diluted product can be stored in the refrigerator for a four-week period without the need for pH adjustment. The use of this product should minimize errors in the measurement of small volumes ranging from 0.05 to 0.15 mL of the original product in most nursery patients. Since phenobarbital has a narrow therapeutic index and its serum concentrations must be routinely monitored, accurate drug administration is crucial to determine optimum doses.[4]

The dilution of the commercially available product would also decrease the volume of propylene glycol administered to infants. Glasgow et al.[2] have reported hyperosmolality in small infants caused by propylene glycol, which is present in multivitamin preparations and drugs including phenobarbital sodium, digoxin, diazepam, vitamin D, and hydralazine. It is not uncommon for a small, sick infant to receive multiple drugs and injectable multivitamins for nutritional support, increasing the risk of propylene glycol toxicity. Other adverse effects of propylene glycol, including central nervous system, renal, hematologic, and cardiac toxicity, have also been reported.[2] The dilution of phenobarbital sodium minimizes these problems.

Conclusion

Phenobarbital sodium can be diluted from the commercially available concentration of 65 mg/mL to a concentration of 10 mg/mL with 0.9% sodium chloride injection and stored over a four-week period in the refrigerator without loss of potency.

References

1. Seay AR, Bray PF. Significance of seizures in infants weighing less than 2,500 grams. *Arch Neurol*. 1977; 34:381.
2. Glasgow AM, Beeckx RL, Miller MK et al. Hyperosmolality in small infants due to propylene glycol. *Pediatrics*. 1985; 72:353–5.
3. The United States pharmacopeia, 20th rev., and The National Formulary, 15th ed. Rockville, MD: The United States Pharmacopeial Convention; 1980:609–10.
4. Roberts PJ. Anticonvulsants in drug therapy in infants: pharmacologic principles and clinical experience. Philadelphia: W.B. Saunders Company; 1984:94–118.

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This paper requesting to schedule and/or conduct an interview is appropriate because:

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Martin Fessenmaier

S-signature:
/Martin Fessenmaier/

Registration Number:
46697

U.S. Application Number:
17025881

Confirmation Number:
8113

E-mail Address:
mfessenmaier@umbergzipser.com

Phone Number:
+1 9497359158

Proposed Time of Interview:
8-24-2021 2:00 PM ET

Alternative Proposed Time(s) of Interview:
8-24-2021 3:00 PM ET

Alternative Proposed Time(s) of Interview:
8-25-2021 1:00 PM ET

Prefered Interview Type:
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I am the applicant or applicant's representative for this application.

Topic for Discussion:
Draft amendments for response to NFOA

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EFS ID:	43592017
Application Number:	17025881
International Application Number:	
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Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
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Receipt Date:	24-AUG-2021
Filing Date:	18-SEP-2020
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Application Type:	Utility under 35 USC 111(a)

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1		Response-03_NFOA_6-1-21_REV001.pdf	162338 018324926c7f74d42076a5936440c2735248f0d37	yes	10

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Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

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Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION

Sir:

This paper responds to the Office Action dated 01 June 2021. Please enter the amendments shown herein.

- Claim amendments begin on page 2.
- Remarks begin on page 6.
- Appendix: An interview Summary is attached to this paper.

AMENDMENTS TO THE CLAIMS

This listing of claims represents the current version of claims before the Office:

1. (currently amended) A pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, wherein the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium is produced by:

   adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 to 9.6; and

   lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with water content at or below 1.1%, wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.

2. (currently amended) A method of producing a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, the method comprising:

   adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 and 9.6 ~~9.2 to 10.2~~; and

   lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with a water content at or below 1.1%, wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.

3. Cancelled.

4. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the water is Water for Injection ~~is~~ sparged with nitrogen prior to adding the Phenobarbital Sodium.

5. (previously presented) The pharmaceutical composition of claim 4, wherein the Water for Injection is sparged with nitrogen for at least 30 minutes prior to adding the Phenobarbital Sodium.

6. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the water is cooled to or between 2 to 8°C prior to adding the Phenobarbital Sodium.
7. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the forming of the Phenobarbital Sodium solution with the pH at or between 9.2 to 10.2 comprises measuring the pH of the Phenobarbital Sodium solution, and wherein if the pH is higher than 10.2, the forming comprises adding Hydrochloric acid (HCl).
8. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.1% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
9. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.05% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
10. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no detectable amount of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
11. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium powder is stable up to at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).
12. (previously presented) The pharmaceutical composition of claim 11, wherein the stability of the lyophilized Phenobarbital Sodium powder is determined by adding the lyophilized Phenobarbital Sodium powder to an aqueous solution to form a reconstituted Phenobarbital Sodium solution and analyzing to quantitate the amount of phenylethylacetylurea (PEAU), 2-ethyl-2-phenylmalonamide (2EPMM), and/or alpha-phenylbutyrylguanidine (PBG) in the reconstituted Phenobarbital Sodium solution.

13. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the Phenobarbital Sodium solution is at a concentration of 65 mg/mL, 100 mg/mL, 130 mg/mL or 200 mg/mL.

14. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising filtering the Phenobarbital Sodium solution prior to lyophilizing.

15. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising:

aliquoting the Phenobarbital Sodium solution into a vial prior to lyophilization; and

after lyophilization, applying a closure to the vial under vacuum.

16. (currently amended) A method of treating an individual in need of Phenobarbital Sodium, the method comprising:

adding saline, water for injection, or a dextrose solution to a composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium as in claim 1 immediately prior to administration to form a Phenobarbital Sodium solution with no more than 0.2% phenylethylacetylurea (PEAU); and

administering the Phenobarbital Sodium solution to the individual,

wherein the efficacy of the treating is not diminished, and the side effects caused by degradation products of the Phenobarbital Sodium are not increased as a consequence of an extended storage duration of the Phenobarbital Sodium powder of at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).

17. (original) The method of claim 16, wherein the individual suffers from epilepsy.

18. (original) The method of claim 17, wherein the individual is a newborn suffering from neonatal epilepsy.

19. (original) The method of claim 16, wherein the composition is a powder dose of 65 mg, 100, 130 mg or 200 mg.

20. (original) The method of claim 16, wherein the administering comprises intramuscular injection or intravenous injection.

21-23. Cancelled

24. (new) The composition as in claim 1, wherein the steps of freezing and evacuation are conducted at a temperature of about -50°C and primary drying is conducted at a temperature increasing from about -50°C to 0°C.
25. (new) The composition as in claim 24, wherein the primary drying is conducted at least during a portion thereof at a temperature increasing from about -45°C to -35°C.
26. (new) The composition as in claim 1, wherein the secondary drying is conducted at least during a portion thereof at temperature between 25°C and 50°C.
27. (new) The composition as in claim 1, wherein the steps of evacuation, primary drying, and secondary drying are conducted at a vacuum of about 75 mTorr.

REMARKS/ARGUMENTS

The Office action dated 01 June 2021 has been received and carefully considered. By this amendment, claims 1, 2, 4, 6-11, and 13-16 are amended. Claims 3, 21-23 are cancelled. New claims 24-27 are added. After entry of this Amendment, claims 1, 2, 4-20, 24-27 will be pending. With these amendments, no new matter is added. In view of these amendments and the following remarks, Applicants respectfully request reconsideration.

Claim Objection

Claim 22 is objected to as being a substantial duplicate of claim 1. Claim 22 is presently cancelled.

35 U.S.C. §103

The Office rejected **claims 1-3, 8-13, 15, and 21-22** under 35 U.S.C. §103 as obvious over Sun in view of Parker and Snyder. In view of the amendments herein, and arguments below, Applicant respectfully disagrees.

As amended, claim 1 incorporates limitations of claim 2 and further narrows the range of pH from a previous range of 9.2 to 10.2 to a reduced range of 9.3 to 9.9.

Responding to previous communication, the Office did not refute the arguments regarding the importance of the properly selected parameters for the method of producing a lyophilized powder of Phenobarbital Sodium. The Office pointed out however, that "*the features upon which applicant relies (i.e., pH in the range of 9.3 to 9.6) are not recited in the rejected claims*" – bottom of page 10 of the Office Action.

The Applicant amended claim 1 to include manufacturing steps of claim 2 including a more restricted range of pH. Claim 1 now specifically recites "*adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 to 9.6*". The range now starts at 9.3, not at 9.2 as supported by the data in the specification. The range ends at 9.9, not 10.2 as supported by examples of further data cited in the specification, for example in Table 2, Table 7, Table 10, and Table 11.

The Office relies on Parker as disclosing the pH in the range from 9 to 12. However, Parker discloses this pH for a liquid solution, not a lyophilized powder composition of Phenobarbital Sodium. Parker explains one mechanism of degradation of Phenobarbital Sodium in the presence of water in paragraph [0004], namely "the presence of the hydroxyl ions in the formulation from the water results in a hydrolysis pathway that can destroy the phenobarbital ring complex." Parker goes on to say that "this destruction results in the possible formation of impurities including harmful degradants or precipitates." As can be appreciated by those skilled in the art, there is little or no hydrolysis occurring in a powder of the present invention as the water is removed during manufacturing steps as described and the moisture content is greatly reduced. Therefore, pH considerations described by Parker are not applicable to the present disclosure and cannot be relied upon for anticipating the present invention.

In addition, Parker discusses using further components of the liquid solution, such as C1-C4 alcohol, propylene glycol, etc. which affect the degradation processes.

Finally, a water based HCl solution is used for pH adjustment in Parker, which further increases water content and a propensity for hydrolysis. The present invention avoids use of HCl for pH adjustment to reduce that risk.

Altogether, Parker does not teach or fairly suggest the present invention as pH of the multi-component liquid has little or no relevance to pH of the Phenobarbital Sodium powder with regard to degradation processes during storage.

Claim 2 is amended to further reduce the range of pH from 9.3 to 9.6 as suggested by the Office.

Claims 4, 6-11, 13-15 are amended to correct the dependency from presently cancelled claim 3 to claim 1.

Claim 16 is amended to be dependent on claim 1 for reasons explained above.

The Office acknowledged statements made in the previous communications with regard to the importance of a particular lyophilization cycle but stated that these specific conditions are not

recited in the claims. In response, the Applicant points to claim 21, which is not specifically addressed in the previous Office Action. The Office has rejected claim 21 together with other claims based on the cited art but failed to provide any reasoned statement with regard to the details of the lyophilization process. Citing Snyder is not sufficient to anticipate claim 21 as Snyder describes a generic lyophilization process and fails to teach or suggest the steps as described in claim 21.

Limitations of claim 21 are now included in independent claim 1, with claim 21 presently cancelled. Claim 1 contains an additional limitation of water content in the dry powder of Phenobarbital Sodium after lyophilization not exceeding 1.1%. Support for this is found in Table 10 and Table 11 (initial water content 0.6%), Table 12 (initial water content 1.1%), Table 13 (initial water content 0.8%), and Table 16 (initial water content 0.7%) – all of these table provide initial (after lyophilization is done) water content of 1.1% and below.

To further support the importance of a particular lyophilization process to produce the desired result of long-term stability of the dry powder, new claims 24-27 provide additional claim limitations by reciting details of the lyophilization process steps as supported by Table 15 of the specification. In view of the amendments and above arguments, Applicant believes that the rejections should be overcome.

Request For Allowance

Claims 1, 2, 4-20, 24-27 are pending in this application. Applicant requests allowance of all pending claims.

Respectfully submitted,
Umberg Zipser LLP

Date: August 24, 2021

By: /Martin Fessenmaier Ph.D./
Martin Fessenmaier Ph.D.
Reg. No. 46697

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INTERVIEW SUMMARY

Following the telephonic interview conducted on August 24, 2021, the Applicant submits the interview summary in accordance to MPEP 713:

(A) Application number: 17/025,881

(B) Name of applicant: Bala Tripura Sundari Chodavarapu.

(C) Name of examiner: Yanzhi ZHANG

(D) Date of interview: May 7, 2019.

(E) Type of interview: The interview was conducted by telephone.

(F) Name of participant(s) (applicant, applicant's representative, etc.): Applicant's representative was Martin Fessenmaier (Patent Agent; Reg.No.46697), Bala Tripura Sundari Chodavarapu (Inventor)

(G) An indication whether or not an exhibit was shown or a demonstration conducted: -/-

(H) An identification of the claims discussed: 1-2, 4-20, and 24-27

(I) An identification of the specific prior art discussed: Sun, Parker, Snyder, all of record

(J) An indication whether an agreement was reached and if so, a description of the general nature of the agreement: The applicant discussed a draft response and the specific nature of the claimed product and process conditions needed to prepare same. The Examiner noted that the proposed amendments and arguments would help advance the case, and Applicant agreed to submit a response accordingly.

Respectfully submitted,
Umberg/Zipser, LLP

Date: August 24, 2021

By /Martin Fessenmaier/
Martin Fessenmaier, Ph.D.
Reg. No. 46697

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To: rdean@umbergzipser.com,patents@umbergzipser.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
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Aug 27, 2021 04:28:10 AM

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Application	Document	Mailroom Date	Attorney Docket No.
17025881	EXIN	08/27/2021	104078.0001US
	OA.APPENDIX	08/27/2021	104078.0001US

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
17/025,881	09/18/2020	Bala Tripura Sundari Chodavarapu	104078.0001US	8113

149344 7590 08/27/2021

Umberg Zipser LLP
1920 Main Street Suite 750
Irvine, CA 92614

EXAMINER	
ZHANG, YANZHI	

ART UNIT	PAPER NUMBER
1617	

NOTIFICATION DATE	DELIVERY MODE
08/27/2021	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patents@umbergzipser.com
rdean@umbergzipser.com

PTOL-90A (Rev. 04/07)

Applicant-Initiated Interview Summary	Application No. 17/025,881	Applicant(s) Chodavarapu et al.		
	Examiner YANZHI ZHANG	Art Unit 1617	AIA (First Inventor to File) Status Yes	Page 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	Title	Type
YANZHI ZHANG	Primary Examiner	Telephonic
Martin Fessenmaier, Ph. D	Attorney of Record	
Dr. Chodavarapu	Inventor	

Date of Interview: 24 August 2021

Issues Discussed:

Proposed Amendment(s)

The proposed amendments (see attached) were discussed.

Dr. Fessenmaier and Dr. Chodavarapu pointed out the distinction between the instantly claimed subject matter and the prior art cited in the office action mailed 06/01/21. The examiner agreed that the proposed amendments would overcome the rejection of record. However, the proposed amendments warranted a new search and consideration.

Should the claimed subject matter found patentable except minor issues, Dr. Fessenmaier and the examiner agreed further communication to resolve the issues.

☑ Attachment

/YANZHI ZHANG/ Primary Examiner, Art Unit 1617	08/24/21

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

DRAFT ONLY – PLEASE DO NOT ENTER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Appl No.:	17/025,881
First Inventor:	Bala Tripura Sundari Chodavarapu
Filing Date:	2020-09-18
Art Unit:	1617
Examiner:	Yanzhi ZHANG
Attorney Docket No.:	104078.0001US
Title	Lyophilized Compositions of Phenobarbital Sodium Salt

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DRAFT RESPONSE TO NON-FINAL OFFICE ACTION

Sir:

This paper responds to the Office Action dated 01 June 2021. Please enter the amendments shown herein.

- Claim amendments begin on page 2.
- Remarks begin on page 6.

DRAFT AMENDMENTS TO THE CLAIMS

This listing of claims represents the current version of claims before the Office:

1. (currently amended) A pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, wherein the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium is produced by:

   adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 to 9.6; and

   lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with water content at or below 1.1%, wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.

2. (currently amended) A method of producing a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, the method comprising:

   adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 and 9.6 ~~9.2 to 10.2~~, and

   lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with a water content at or below 1.1%, wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0 °C, followed by evacuating and secondary drying at a stable or declining temperature above 0 °C.

3. Cancelled.

4. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the water is Water for Injection ~~is~~ sparged with nitrogen prior to adding the Phenobarbital Sodium.

5. (previously presented) The pharmaceutical composition of claim 4, wherein the Water for Injection is sparged with nitrogen for at least 30 minutes prior to adding the Phenobarbital Sodium.

6. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the water is cooled to or between 2 to 8°C prior to adding the Phenobarbital Sodium.
7. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the forming of the Phenobarbital Sodium solution with the pH at or between 9.2 to 10.2 comprises measuring the pH of the Phenobarbital Sodium solution, and wherein if the pH is higher than 10.2, the forming comprises adding Hydrochloric acid (HCl).
8. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.1% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
9. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no more than 0.05% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
10. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium forms no detectable amount of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).
11. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the lyophilized Phenobarbital Sodium powder is stable up to at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).
12. (previously presented) The pharmaceutical composition of claim 11, wherein the stability of the lyophilized Phenobarbital Sodium powder is determined by adding the lyophilized Phenobarbital Sodium powder to an aqueous solution to form a reconstituted Phenobarbital Sodium solution and analyzing to quantitate the amount of phenylethylacetylurea (PEAU), 2-ethyl-2-phenylmalonamide (2EPMM), and/or alpha-phenylbutyrylguanidine (PBG) in the reconstituted Phenobarbital Sodium solution.

13. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein the Phenobarbital Sodium solution is at a concentration of 65 mg/mL, 100 mg/mL, 130 mg/mL or 200 mg/mL.

14. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising filtering the Phenobarbital Sodium solution prior to lyophilizing.

15. (currently amended) The pharmaceutical composition of claim 1 ~~3~~, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising:

aliquoting the Phenobarbital Sodium solution into a vial prior to lyophilization; and

after lyophilization, applying a closure to the vial under vacuum.

16. (currently amended) A method of treating an individual in need of Phenobarbital Sodium, the method comprising:

adding saline, water for injection, or a dextrose solution to a composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium as in claim 1 immediately prior to administration to form a Phenobarbital Sodium solution with no more than 0.2% phenylethylacetylurea (PEAU); and

administering the Phenobarbital Sodium solution to the individual,

wherein the efficacy of the treating is not diminished, and the side effects caused by degradation products of the Phenobarbital Sodium are not increased as a consequence of an extended storage duration of the Phenobarbital Sodium powder of at least 6 months at accelerated stability conditions (ACC) which is equivalent to 24 months at room temperature (RT).

17. (original) The method of claim 16, wherein the individual suffers from epilepsy.

18. (original) The method of claim 17, wherein the individual is a newborn suffering from neonatal epilepsy.

19. (original) The method of claim 16, wherein the composition is a powder dose of 65 mg, 100, 130 mg or 200 mg.

20. (original) The method of claim 16, wherein the administering comprises intramuscular injection or intravenous injection.

21-23. Cancelled

24. (new) The composition as in claim 1, wherein the steps of freezing and evacuation are conducted at a temperature of about -50°C and primary drying is conducted at a temperature increasing from about -50°C to 0°C.
25. (new) The composition as in claim 24, wherein the primary drying comprises a segment of drying at a temperature increasing from about -45°C to -35°C.
26. (new) The composition as in claim 1, wherein the secondary drying comprises a segment of drying at temperature between 25°C and 50°C.
27. (new) The composition as in claim 1, wherein the steps of evacuation, primary drying, and secondary drying are conducted at a vacuum of about 75 mTorr.

DRAFT REMARKS/ARGUMENTS

The Office action dated 01 June 2021 has been received and carefully considered. By this amendment, claims 1, 2, 4, 6-11, and 13-16 are amended. Claims 3, 21-23 are cancelled. New claims 24-27 are added. After entry of this Amendment, claims 1, 2, 4-20, 24-27 will be pending. With these amendments, no new matter is added. In view of these amendments and the following remarks, Applicants respectfully request reconsideration.

Claim Objection

Claim 22 is objected to as being a substantial duplicate of claim 1. Claim 22 is presently cancelled.

35 U.S.C. §103

The Office rejected **claims 1-3, 8-13, 15, and 21-22** under 35 U.S.C. §103 as obvious over Sun in view of Parker and Snyder. In view of the amendments herein, and arguments below, Applicant respectfully disagrees.

As amended, claim 1 incorporates limitations of claim 2 and further narrows the range of pH from a previous range of 9.2 to 10.2 to a reduced range of 9.3 to 9.9.

Responding to previous communication, the Office did not refute the arguments regarding the importance of the properly selected parameters for the method of producing a lyophilized powder of Phenobarbital Sodium. The Office pointed out however, that "*the features upon which applicant relies (i.e., pH in the range of 9.3 to 9.6) are not recited in the rejected claims*" – bottom of page 10 of the Office Action.

The Applicant amended claim 1 to include manufacturing steps of claim 2 including a more restricted range of pH. Claim 1 now specifically recites "*adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 to 9.9*". The range now starts at 9.3, not at 9.2 as supported by the data in the specification. The range ends at 9.9, not 10.2 as supported by examples of further data cited in the specification, for example in Table 2, Table 7, Table 10, and Table 11.

The Office relies on Parker as disclosing the pH in the range from 9 to 12. However, Parker discloses this pH for a liquid solution, not a lyophilized powder composition of Phenobarbital Sodium. Parker explains one mechanism of degradation of Phenobarbital Sodium in the presence of water in paragraph [0004], namely "the presence of the hydroxyl ions in the formulation from the water results in a hydrolysis pathway that can destroy the phenobarbital ring complex." Parker goes on to say that "this destruction results in the possible formation of impurities including harmful degradants or precipitates." As can be appreciated by those skilled in the art, there is little or no hydrolysis occurring in a powder of the present invention as the water is removed during manufacturing steps as described and the moisture content is greatly reduced. Therefore, pH considerations described by Parker are not applicable to the present disclosure and cannot be relied upon for anticipating the present invention.

In addition, Parker discusses using further components of the liquid solution, such as C1-C4 alcohol, propylene glycol, etc. which affect the degradation processes.

Finally, a water based HCl solution is used for pH adjustment in Parker, which further increases water content and a propensity for hydrolysis. The present invention avoids use of HCl for pH adjustment to reduce that risk.

Altogether, Parker does not teach or fairly suggest the present invention as pH of the multi-component liquid has little or no relevance to pH of the Phenobarbital Sodium powder with regard to degradation processes during storage.

Claim 2 is amended to further reduce the range of pH from 9.3 to 9.6 as suggested by the Office.

Claims 4, 6-11, 13-15 are amended to correct the dependency from presently cancelled claim 3 to claim 1.

Claim 16 is amended to be dependent on claim 1 for reasons explained above.

The Office acknowledged statements made in the previous communications with regard to the importance of a particular lyophilization cycle but stated that these specific conditions are not

recited in the claims. In response, the Applicant points to claim 21, which is not specifically addressed in the previous Office Action. The Office has rejected claim 21 together with other claims based on the cited art but failed to provide any reasoned statement with regard to the details of the lyophilization process. Citing Snyder is not sufficient to anticipate claim 21 as Snyder describes a generic lyophilization process and fails to teach or suggest the steps as described in claim 21.

Limitations of claim 21 are now included in independent claim 1, with claim 21 presently cancelled. Claim 1 contains an additional limitation of water content in the dry powder of Phenobarbital Sodium after lyophilization not exceeding 1.1%. Support for this is found in Table 10 and Table 11 (initial water content 0.6%), Table 12 (initial water content 1.1%), Table 13 (initial water content 0.8%), and Table 16 (initial water content 0.7%) – all of these table provide initial (after lyophilization is done) water content of 1.1% and below.

To further support the importance of a particular lyophilization process to produce the desired result of long-term stability of the dry powder, new claims 24-27 provide additional claim limitations by reciting details of the lyophilization process steps as supported by Table 15 of the specification.

Request For Allowance

Claims 1, 2, 4-20, 24-27 are pending in this application. Applicant requests allowance of all pending claims.

Respectfully submitted,
Umberg Zipser LLP

Date: August 24, 2021

By: /Martin Fessenmaier Ph.D./
Martin Fessenmaier Ph.D.
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Electronic Acknowledgement Receipt	
EFS ID:	43650673
Application Number:	17025881
International Application Number:	
Confirmation Number:	8113
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
Customer Number:	149344
Filer:	Jay Philip Lessler
Filer Authorized By:	
Attorney Docket Number:	104078.0001US
Receipt Date:	31-AUG-2021
Filing Date:	18-SEP-2020
Time Stamp:	16:03:54
Application Type:	

Payment information:

Submitted with Payment	yes
Payment Type	CARD
Payment was successfully received in RAM	$180
RAM confirmation Number	E20218UG03494653
Deposit Account	232185
Authorized User	Jay Lessler
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37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

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1	Non Patent Literature	11A.pdf	1070044 b01d9a9caf5cdd1a32e20424bfc53122cc9b d84f1	no	3
Warnings:					
Information:					
2	Non Patent Literature	12.pdf	419353 6c4abd4f68f2db3c4e468be44eff88e15750 e9e1	no	2
Warnings:					
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3	Non Patent Literature	13.pdf	257059 31b11bfe7b19456108d30d63a685196344 b6640	no	2
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4	Non Patent Literature	17.PDF	483812 ecf45db562bcbe6b3ce4bf8518a631d65e2 d9694	no	11
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5	Non Patent Literature	18.PDF	960249 3e7bbcf707f4f106e0d8fa82b2b12710e395 2670	no	49
Warnings:					
Information:					
6	Non Patent Literature	19.pdf	2613594 913ed21a0690508f4c589bd10697685114c0 6f842	no	5
Warnings:					
Information:					

7	Non Patent Literature	20.pdf	3812371 a1ca8a3117ae6fa80818728 1f4e160996012 c3b3	no	16
Warnings:					
Information:					
8	Non Patent Literature	15A.pdf	14460443 c3669c37b099fb7ef03d926cc60b6ba708f6 64df	no	22
Warnings:					
Information:					
9	Concise Description of Relevance	Concise-description-generated.pdf	47994 1a39e4574471233cb9889a19cceb556d65d 82c2a	no	9
Warnings:					
Information:					
10	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	71232 d653d5d23ba78913bede0de4e61877dd9 8254bc	no	5
Warnings:					
Information:					
11	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	23756 996ad809a852c9902fafd971212790c1e239 92da	no	1
Warnings:					
Information:					
12	Fee Worksheet (SB06)	fee-info.pdf	37676 0548f4d947e44aa6e086d09be84aff03f58f c02a	no	2
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			Total Files Size (in bytes):	24257583	

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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Doc Code: 3P.RELEVANCE
Document Description: Concise Description of Relevance

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	17025881

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance
1	8436190	See attached

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance
1	20170143719	See attached

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Encyclopedia of Pharmaceutical Technology (2007), Vol 1, Third Edition; James Swarbrick; Freeze, Freeze Drying, Scale-Up Considerations (Pg. 1807-1874)	See attached

2	R. Sandhyarani, Development and Evaluation of Lyophilized Product of Apo-Acetozolamide, IOSR Journal of Pharmacy; Vol. 6, Issue 9 Version. 1, pp. 48-63, Sept. 2016	See attached

3	I.B. Butler, et al., Removal of dissolved oxygen from water: a comparison of four common techniques, Talanta, Vol. 41, No. 2, pp. 211-215, 1994	See attached

4	Entry from OhioLINK database for Kern, https://etd.ohiolink.edu/apexprod/-rws_olink/r/1501/10?clear=10&p10_accession_num=osu1486476025025146 (accessed on July 14, 2021)	See attached

5	OhioLINK: Frequently Asked Questions, https://etd.ohiolink.edu/apexprod/-f?p=1501:2:310637464866699 (accessed on July 14, 2021)	See attached

6	D.S. Przic, et al., Lyophilization - The Process and Industrial Scale, Chem. Ind. Vol. 58, No. 12, pp. 552-562, 2004	See attached

7	Lyophilization of Parenteral (7/93) (LoP) (https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-guides/lyophilization-parenteral-793) (dated November 11, 2014) (accessed on July 19, 2021)	See attached

8	Access to Ohio State Dissertations and Theses, https://gradsch.osu.edu/access-distribution (accessed on July 14, 2021)	See attached

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

U.S. PATENTS				
Cite No	Patent Number	Kind Code[1]	Issue Date (YYYY-MM-DD)	First Named Inventor
1	8436190	B2	2013-05-07	Jason Brittain

U.S. PATENT APPLICATION PUBLICATIONS				
Cite No	Publication Number	Kind Code[1]	Publication Date (YYYY-MM-DD)	First Named Inventor
1	20170143719	A1	2017-05-25	Michael G. Parker

FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
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NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

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3	D.S. Przic, et al., Lyophilization - The Process and Industrial Scale, Chem. Ind. Vol. 58, No. 12, pp. 552-562, 2004	☐	☐

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4	OhioLINK: Frequently Asked Questions, https://etd.ohiolink.edu/apexprod/-f?p=1501:2:310637464866699 (accessed on July 14, 2021)	☐	☐
5	Entry from OhioLINK database for Kern, https://etd.ohiolink.edu/apexprod/-rws_olink/r/1501/10?clear=10&p10_accession_num=osu1486476025025146 (accessed on July 14, 2021)	☐	☐
6	I.B. Butler, et al., Removal of dissolved oxygen from water: a comparison of four common techniques, Talanta, Vol. 41, No. 2, pp. 211-215, 1994	☐	☐

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8	Encyclopedia of Pharmaceutical Technology (2007), Vol 1, Third Edition; James Swarbrick; Freeze, Freeze Drying, Scale-Up Considerations (Pg. 1807-1874)	☐	☐

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☒ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☐ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

Signature	/Jay P, Lessler/		
Name/Print	Jay P. Lessler	Registration Number (if applicable)	41151

Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

Other Prior Art

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8. https://gradforms.osu.edu/
9. tel:614-247-7292
0. mailto:watson.11@osu.edu

Lyophilization of Parenteral (7/93)

GUIDE TO INSPECTIONS OF LYOPHILIZATION OF PARENTERALS

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INTRODUCTION

Lyophilization or freeze drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from solid to vapor without passing through a liquid phase. The process consists of three separate, unique, and interdependent processes; freezing, primary drying (sublimation), and secondary drying (desorption).

The advantages of lyophilization include:

Ease of processing a liquid, which simplifies aseptic handling

Enhanced stability of a dry powder

Removal of water without excessive heating of the product

Enhanced product stability in a dry state

Rapid and easy dissolution of reconstituted product

Disadvantages of lyophilization include:

Increased handling and processing time

Need for sterile diluent upon reconstitution

Cost and complexity of equipment

The lyophilization process generally includes the following steps:

- Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
- Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
- Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.

- Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
- Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
- Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
- Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizers.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. In order to provide guidance and information to investigators, some industry procedures and deficiencies associated with lyophilized products are identified in this Inspection Guide.

It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product. This discussion will address some of the problems associated with the manufacture and control of a lyophilized dosage form.

PRODUCT TYPE/FORMULATION

Products are manufactured in the lyophilized form due to their instability when in solution. Many of the antibiotics, such as some of the semi-synthetic penicillins, cephalosporins, and also some of the salts of erythromycin, doxycycline and chloramphenicol are made by the lyophilization process. Because they are antibiotics, low bioburden of these formulations would be expected at the time of batching. However, some of the other dosage forms that are lyophilized, such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and many of the biotechnology derived products, have no antibacterial effect when in solution.

For these types of products, bioburden should be minimal and the bioburden should be determined prior to sterilization of these bulk solutions prior to filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). The concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of lyophilized products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be held for any length of time prior to sterilization.

In some cases, manufacturers have performed bioburden testing on bulk solutions after prefiltration and prior to final filtration. While the testing of such solutions may be meaningful in determining the bioburden for sterilization, it does not provide any information regarding the potential formation or presence of endotoxins. While the testing of 0.1 ml samples by LAL methods of bulk solution for endotoxins is of value, testing of at least 100 ml size samples prior to prefiltration, particularly for the presence of gram negative organisms, would be of greater value in evaluating the process. For example, the presence of Pseudomonas sp. in the bioburden of a bulk solution has been identified as an objectionable condition.

FILLING

The filling of vials that are to be lyophilized has some problems that are somewhat unique. The stopper is placed on top of the vial and is ultimately seated in the lyophilizer. As a result the contents of the vial are subject to contamination until they are actually sealed.

Validation of filling operations should include media fills and the sampling of critical surfaces and air during active filling (dynamic conditions).

Because of the active involvement of people in filling and aseptic manipulations, an environmental program should also include an evaluation of microbiological levels on people working in aseptic processing areas. One method of evaluation of the training of operators working in aseptic processing facilities includes the surface monitoring of gloves and/or gowns on a daily basis. Manufacturers are actively sampling the surfaces of personnel working in aseptic processing areas. A reference which provides for this type of monitoring is the USP XXII discussion of the Interpretation of Sterility Test Results. It states under the heading of "Interpretation of Quality Control Tests" that review consideration should be paid to environmental control data, including...microbial monitoring, records of operators, gowns, gloves, and garbing practices. In those situations in which manufacturers have failed to perform some type of personnel monitoring, or monitoring has shown unacceptable levels of contamination, regulatory situations have resulted.

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been noted which utilize an operator to place each stopper on top of the vial by hand. At this time, it would seem that it would be difficult for a manufacturer to justify a hand-stoppering operation, even if sterile forceps are employed, in any type of operation other than filling a clinical batch or very small number of units. Significant regulatory situations have resulted when some manufacturers have hand-stoppered vials. Again, the concern is the immediate avenue of contamination offered by the operator. It is well recognized that people are the major source of contamination in an aseptic processing filling operation. The longer a person works in an aseptic operation, the more microorganisms will be shed and the greater the probability of contamination.

Once filled and partially stoppered, vials are transported and loaded into the lyophilizer. The transfer and handling, such as loading of the lyophilizer, should take place under primary barriers, such as the laminar flow hoods under which the vials were filled. Validation of this handling should also include the use media fills.

Regarding the filling of sterile media, there are some manufacturers who carry out a partial lyophilization cycle and freeze the media. While this could seem to greater mimic the process, the freezing of media could reduce microbial levels of some contaminants. Since the purpose of the media fill is to evaluate and justify the aseptic capabilities of the process, the people and the system, the possible reduction of microbiological levels after aseptic manipulation by freezing would not be warranted. The purpose of a media fill is not to determine the lethality of freezing and its effect on any microbial contaminants that might be present.

In an effort to identify the particular sections of filling and aseptic manipulation that might introduce contamination, several manufacturers have resorted to expanded media fills. That is, they have filled approximately 9000 vials during a media fill and segmented the fill into three stages. One stage has included filling of 3000 vials and stoppering on line; another stage included filling 3000 vials, transportation to the lyophilizer and then stoppering; a third stage included the filling of 3000 vials, loading in the lyophilizer, and exposure to a portion of the nitrogen flush and then stoppering. Since lyophilizer sterilization and sterilization of the nitrogen system used to backfill require separate validation, media fills should primarily validate the filling, transportation and loading aseptic operations.

The question of the number of units needed for media fills when the capacity of the process is less than 3000 units is frequently asked, particularly for clinical products. Again, the purpose of the media fill is to assure that product can be aseptically processed without contamination under operating conditions. It would seem, therefore, that the maximum number of units of media filled be equivalent to the maximum batch size if it is less than 3000 units.

After filling, dosage units are transported to the lyophilizer by metal trays. Usually, the bottom of the trays are removed after the dosage units are loaded into the lyophilizer. Thus, the dosage units lie directly on the lyophilizer shelf. There have been some situations in which manufacturers have loaded the dosage units on metal trays which were not removed. Unfortunately, at one manufacturer, the trays warped which caused a moisture problem in some dosage units in a batch.

In the transport of vials to the lyophilizer, since they are not sealed, there is concern for the potential for contamination. During inspections and in the review of new facilities, the failure to provide laminar flow coverage or a primary barrier for the transport and loading areas of a lyophilizer has been regarded as an objectionable condition. One manufacturer as a means of

correction developed a laminar flow cart to transport the vials from the filling line to the lyophilizer. Other manufacturers building new facilities have located the filling line close to the lyophilizer and have provided a primary barrier extending from the filling line to the lyophilizer.

In order to correct this type of problem, another manufacturer installed a vertical laminar flow hood between the filling line and lyophilizer. Initially, high velocities with inadequate return caused a contamination problem in a media fill. It was speculated that new air currents resulted in rebound contamination off the floor. Fortunately, media fills and smoke studies provided enough meaningful information that the problem could be corrected prior to the manufacture of product. Typically, the lyophilization process includes the stoppering of vials in the chamber.

Another major concern with the filling operation is assurance of fill volumes. Obviously, a low fill would represent a subpotency in the vial. Unlike a powder or liquid fill, a low fill would not be readily apparent after lyophilization particularly for a biopharmaceutical drug product where the active ingredient may be only a milligram. Because of the clinical significance, sub-potency in a vial potentially can be a very serious situation.

For example, in the inspection of a lyophilization filling operation, it was noted that the firm was having a filling problem. The gate on the filling line was not coordinated with the filling syringes, and splashing and partial filling was occurring. It was also observed that some of the partially filled vials were loaded into the lyophilizer. This resulted in rejection of the batch.

On occasion, it has been seen that production operators monitoring fill volumes record these fill volumes only after adjustments are made. Therefore, good practice and a good quality assurance program would include the frequent monitoring of the volume of fill, such as every 15 minutes. Good practice would also include provisions for the isolation of particular sections of filling operations when low or high fills are encountered.

There are some atypical filling operations which have not been discussed. For example, there have also been some situations in which lyophilization is performed on trays of solution rather than in vials. Based on the current technology available, it would seem that for a sterile product, it would be difficult to justify this procedure.

The dual chamber vial also presents additional requirements for aseptic manipulations. Media fills should include the filling of media in both chambers. Also, the diluent in these vials should contain a preservative. (Without a preservative, the filling of diluent would be analogous to the filling of media. In such cases, a 0% level of contamination would be expected.)

LYOPHILIZATION CYCLE AND CONTROLS

After sterilization of the lyophilizer and aseptic loading, the initial step is freezing the solution. In some cycles, the shelves are at the temperature needed for freezing, while for other cycles, the product is loaded and then the shelves are taken to the freezing temperature necessary for

product freeze. In those cycles in which the shelves are precooled prior to loading, there is concern for any ice formation on shelves prior to loading. Ice on shelves prior to loading can cause partial or complete stoppering of vials prior to lyophilization of the product. A recent field complaint of a product in solution and not lyophilized was attributed to preliminary stoppering of a few vials prior to exposure to the lyophilization cycle. Unfortunately, the firm's 100% vial inspection failed to identify the defective vial.

Typically, the product is frozen at a temperature well below the eutectic point.

The scale-up and change of lyophilization cycles, including the freezing procedures, have presented some problems. Studies have shown the rate and manner of freezing may affect the quality of the lyophilized product. For example, slow freezing leads to the formation of larger ice crystals. This results in relatively large voids, which aid in the escape of water vapor during sublimation. On the other hand, slow freezing can increase concentration shifts of components. Also, the rate and manner of freezing has been shown to have an affect on the physical form (polymorph) of the drug substance.

It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4-5o below the eutectic point. Obviously, the manufacturer should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include: shelf temperature, product temperature, condenser temperature, chamber pressure and condenser pressure. The manufacturing directions should provide for time, temperature and pressure limits necessary for a lyophilization cycle for a product. The monitoring of product temperature is particularly important for those cycles for which there are atypical operating procedures, such as power failures or equipment breakdown.

Electromechanical control of a lyophilization cycle has utilized cam-type recorder-controllers. However, newer units provide for microcomputer control of the freeze drying process. A very basic requirement for a computer controlled process is a flow chart or logic. Typically, operator involvement in a computer controlled lyophilization cycle primarily occurs at the beginning. It consists of loading the chamber, inserting temperature probes in product vials, and entering cycle parameters such as shelf temperature for freezing, product freeze temperature, freezing soak time, primary drying shelf temperature and cabinet pressure, product temperature for establishment of fill vacuum, secondary drying shelf temperature, and secondary drying time.

In some cases, manufacturers have had to continuously make adjustments in cycles as they were being run. In these situations, the lyophilization process was found to be non-validated.

Validation of the software program of a lyophilizer follows the same criteria as that for other processes. Basic concerns include software development, modifications and security. The Guide to Inspection of Computerized Systems in Drug Processing contains a discussion on potential problem areas relating to computer systems. A Guide to the Inspection of Software Development Activities is a reference that provides a more detailed review of software requirements.

Leakage into a lyophilizer may originate from various sources. As in any vacuum chamber, leakage can occur from the atmosphere into the vessel itself. Other sources are media employed within the system to perform the lyophilizing task. These would be the thermal fluid circulated through the shelves for product heating and cooling, the refrigerant employed inside the vapor condenser cooling surface and oil vapors that may migrate back from the vacuum pumping system.

Any one, or a combination of all, can contribute to the leakage of gases and vapors into the system. It is necessary to monitor the leak rate periodically to maintain the integrity of the system. It is also necessary, should the leak rate exceed specified limits, to determine the actual leak site for purposes of repair.

Thus, it would be beneficial to perform a leak test at some time after sterilization, possibly at the beginning of the cycle or prior to stoppering. The time and frequency for performing the leak test will vary and will depend on the data developed during the cycle validation. The pressure rise found acceptable at validation should be used to determine the acceptable pressure rise during production. A limit and what action is to be taken if excessive leakage is found should be addressed in some type of operating document.

In order to minimize oil vapor migration, some lyophilizers are designed with a tortuous path between the vacuum pump and chamber. For example, one fabricator installed an oil trap in the line between the vacuum pump and chamber in a lyophilizer with an internal condenser. Leakage can also be identified by sampling surfaces in the chamber after lyophilization for contaminants. One could conclude that if contamination is found on a chamber surface after lyophilization, then dosage units in the chamber could also be contaminated. It is a good practice as part of the validation of cleaning of the lyophilization chamber to sample the surfaces both before and after cleaning.

Because of the lengthy cycle runs and strain on machinery, it is not unusual to see equipment malfunction or fail during a lyophilization cycle. There should be provisions in place for the corrective action to be taken when these atypical situations occur. In addition to documentation of the malfunction, there should be an evaluation of the possible effects on the product (e.g., partial or complete meltback. Refer to subsequent discussion). Merely testing samples after the

lyophilization cycle is concluded may be insufficient to justify the release of the remaining units. For example, the leakage of chamber shelf fluid into the chamber or a break in sterility would be cause for rejection of the batch.

The review of Preventive Maintenance Logs, as well as Quality Assurance Alert Notices, Discrepancy Reports, and Investigation Reports will help to identify problem batches when there are equipment malfunctions or power failures. It is recommended that these records be reviewed early in the inspection.

CYCLE VALIDATION

Many manufacturers file (in applications) their normal lyophilization cycles and validate the lyophilization process based on these cycles. Unfortunately, such data would be of little value to substantiate shorter or abnormal cycles. In some cases, manufacturers are unaware of the eutectic point. It would be difficult for a manufacturer to evaluate partial or abnormal cycles without knowing the eutectic point and the cycle parameters needed to facilitate primary drying.

Scale-up for the lyophilized product requires a knowledge of the many variables that may have an effect on the product. Some of the variables would include freezing rate and temperature ramping rate. As with the scale-up of other drug products, there should be a development report that discusses the process and logic for the cycle. Probably more so than any other product, scale-up of the lyophilization cycle is very difficult.

There are some manufacturers that market multiple strengths, vial sizes and have different batch sizes. It is conceivable and probable that each will have its own cycle parameters. A manufacturer that has one cycle for multiple strengths of the same product probably has done a poor job of developing the cycle and probably has not adequately validated their process. Investigators should review the reports and data that support the filed lyophilization cycle.

LYOPHILIZER STERILIZATION/DESIGN

The sterilization of the lyophilizer is one of the more frequently encountered problems noted during inspections. Some of the older lyophilizers cannot tolerate steam under pressure, and sterilization is marginal at best. These lyophilizers can only have their inside surfaces wiped with a chemical agent that may be a sterilant but usually has been found to be a sanitizing agent. Unfortunately, piping such as that for the administration of inert gas (usually nitrogen) and sterile air for backfill or vacuum break is often inaccessible to such surface "sterilization" or treatment. It would seem very difficult for a manufacturer to be able to demonstrate satisfactory validation of sterilization of a lyophilizer by chemical "treatment".

Another method of sterilization that has been practiced is the use of gaseous ethylene oxide. As with any ethylene oxide treatment, humidification is necessary. Providing a method for introducing the sterile moisture with uniformity has been found to be difficult.

A manufacturer has been observed employing Water For Injection as a final wash or rinse of the lyophilizer. While the chamber was wet, it was then ethylene oxide gas sterilized. As discussed above, this may be satisfactory for the chamber but inadequate for associated plumbing.

Another problem associated with ethylene oxide is the residue. One manufacturer had a common ethylene oxide/nitrogen supply line to a number of lyophilizers connected in parallel to the system. Thus, there could be some ethylene oxide in the nitrogen supply line during the backfilling step. Obviously, this type of system is objectionable.

A generally recognized acceptable method of sterilizing the lyophilizer is through the use of moist steam under pressure. Sterilization procedures should parallel that of an autoclave, and a typical system should include two independent temperature sensing systems. One would be used to control and record temperatures of the cycle as with sterilizers, and the other would be in the cold spot of the chamber. As with autoclaves, lyophilizers should have drains with atmospheric breaks to prevent back siphonage.

As discussed, there should also be provisions for sterilizing the inert gas or air and the supply lines. Some manufacturers have chosen to locate the sterilizing filters in a port of the chamber. The port is steam sterilized when the chamber is sterilized, and then the sterilizing filter, previously sterilized, is aseptically connected to the chamber. Some manufacturers have chosen to sterilize the filter and downstream piping to the chamber in place. Typical sterilization-in-place of filters may require steaming of both to obtain sufficient temperatures. In this type of system, there should be provisions for removing and/or draining condensate. The failure to sterilize nitrogen and air filters and the piping downstream leading into the chamber has been identified as a problem on a number of inspections.

Since these filters are used to sterilize inert gas and/or air, there should be some assurance of their integrity. Some inspections have disclosed a lack of integrity testing of the inert gas and/or air filter. The question is frequently asked how often should the vent filter be tested for integrity? As with many decisions made by manufacturers, there is a level of risk associated with the operation, process or system, which only the manufacturer can decide. If the sterilizing filter is found to pass the integrity test after several uses or batches, then one could claim its integrity for the previous batches. However, if it is only tested after several batches have been processed and if found to fail the integrity test, then one could question the sterility of all of the previous batches processed. In an effort to minimize this risk, some manufacturers have resorted to redundant filtration.

For most cycles, stoppering occurs within the lyophilizer. Typically, the lyophilizer has some type of rod or rods (ram) which enter the immediate chamber at the time of stoppering. Once the rod enters the chamber, there is the potential for contamination of the chamber. However, since the vials are stoppered, there is no avenue for contamination of the vials in the chamber

which are now stoppered. Generally, lyophilizers should be sterilized after each cycle because of the potential for contamination of the shelf support rods. Additionally, the physical act of removing vials and cleaning the chamber can increase levels of contamination.

In some of the larger units, the shelves are collapsed after sterilization to facilitate loading. Obviously, the portions of the ram entering the chamber to collapse the shelves enters from a non-sterile area. Attempts to minimize contamination have included wiping the ram with a sanitizing agent prior to loading. Control aspects have included testing the ram for microbiological contamination, testing it for residues of hydraulic fluid, and testing the fluid for its bacteriostatic effectiveness. One lyophilizer fabricator has proposed developing a flexible "skirt" to cover the ram.

In addition to microbiological concerns with hydraulic fluid, there is also the concern with product contamination.

During steam sterilization of the chamber, there should be space between shelves that permit passage of free flowing steam. Some manufacturers have placed "spacers" between shelves to prevent their total collapse. Others have resorted to a two phase sterilization of the chamber. The initial phase provides for sterilization of the shelves when they are separated. The second phase provides for sterilization of the chamber and piston with the shelves collapsed.

Typically, biological indicators are used in lyophilizers to validate the steam sterilization cycle. One manufacturer of a Biopharmaceutical product was found to have a positive biological indicator after sterilization at 1210C for 45 minutes. During the chamber sterilization, trays used to transport vials from the filling line to the chamber were also sterilized. The trays were sterilized in an inverted position on shelves in the chamber. It is believed that the positive biological indicator is the result of poor steam penetration under these trays.

The sterilization of condensers is also a major issue that warrants discussion. Most of the newer units provide for the capability of sterilization of the condenser along with the chamber, even if the condenser is external to the chamber. This provides a greater assurance of sterility, particularly in those situations in which there is some equipment malfunction and the vacuum in the chamber is deeper than in the condenser.

Malfunctions that can occur, which would indicate that sterilization of the condenser is warranted, include vacuum pump breakdown, refrigeration system failures and the potential for contamination by the large valve between the condenser and chamber. This is particularly true for those units that have separate vacuum pumps for both the condenser and chamber. When there are problems with the systems in the lyophilizer, contamination could migrate from the condenser back to the chamber. It is recognized that the condenser is not able to be sterilized in many of the older units, and this represents a major problem, particularly in those cycles in which there is some equipment and/or operator failure.

As referenced above, leakage during a lyophilization cycle can occur, and the door seal or gasket presents an avenue of entry for contaminants. For example, in an inspection, it was noted that during steam sterilization of a lyophilizer, steam was leaking from the unit. If steam could leak from a unit during sterilization, air could possibly enter the chamber during lyophilization.

Some of the newer lyophilizers have double doors - one for loading and the other for unloading. The typical single door lyophilizer opens in the clean area only, and contamination between loads would be minimal. This clean area, previously discussed, represents a critical processing area for a product made by aseptic processing. In most units, only the piston raising/lowering shelves is the source of contamination. For a double door system unloading the lyophilizer in a non-sterile environment, other problems may occur. The non-sterile environment presents a direct avenue of contamination of the chamber when unloading, and door controls similar to double door sterilizers should be in place.

Obviously, the lyophilizer chamber is to be sterilized between batches because of the direct means of contamination. A problem which may be significant is that of leakage through the door seal. For the single door unit, leakage prior to stoppering around the door seal is not a major problem from a sterility concern, because single door units only open into sterile areas. However, leakage from a door gasket or seal from a non-sterile area would present a significant microbiological problem. In order to minimize the potential for contamination, it is recommended that the lyophilizers be unloaded in a clean room area to minimize contamination. For example, in an inspection of a new manufacturing facility, it was noted that the unloading area for double door units was a clean room, with the condenser located below the chamber on a lower level.

After steam sterilization, there is often some condensate remaining on the floor of the chamber. Some manufacturers remove this condensate through the drain line while the chamber is still pressurized after sterilization. Unfortunately, some manufacturers have allowed the chamber to come to and remain at atmospheric pressure with the drain line open. Thus, non-sterile air could contaminate the chamber through the drain line. Some manufacturers have attempted to dry the chamber by blowing sterile nitrogen gas through the chamber at a pressure above atmospheric pressure.

In an inspection of a biopharmaceutical drug product, a Pseudomonas problem probably attributed to condensate after sterilization was noted. On a routine surface sample taken from a chamber shelf after sterilization and processing, a high count of Pseudomonas sp. was obtained. After sterilization and cooling when the chamber door was opened, condensate routinely spilled onto the floor from the door. A surface sample taken from the floor below the door also revealed Pseudomonas sp. contamination. Since the company believed the condensate remained in the chamber after sterilization, they repiped the chamber drain and added a line to a water seal vacuum pump.

FINISHED PRODUCT TESTING FOR

LYOPHILIZED PRODUCTS

There are several aspects of finished product testing which are of concern to the lyophilized dosage form. These include dose uniformity testing, moisture and stability testing, and sterility testing.

(a) Dose Uniformity

The USP includes two types of dose uniformity testing: content uniformity and weight variation. It states that weight variation may be applied to solids, with or without added substances, that have been prepared from true solutions and freeze-dried in final containers. However, when other excipients or other additives are present, weight variation may be applied, provided there is correlation with the sample weight and potency results. For example, in the determination of potency, it is sometimes common to reconstitute and assay the entire contents of a vial without knowing the weight of the sample. Performing the assay in this manner will provide information on the label claim of a product, but without knowing the sample weight will provide no information about dose uniformity. One should correlate the potency result obtained form the assay with the weight of the sample tested.

(b) Stability Testing

An obvious concern with the lyophilized product is the amount of moisture present in vials. The manufacturer's data for the establishment of moisture specifications for both product release and stability should be reviewed. As with other dosage forms, the expiration date and moisture limit should be established based on worst case data. That is, a manufacturer should have data that demonstrates adequate stability at the moisture specification.

As with immediate release potency testing, stability testing should be performed on vials with a known weight of sample. For example, testing a vial (sample) which had a higher fill weight (volume) than the average fill volume of the batch would provide a higher potency results and not represent the potency of the batch. Also, the expiration date and stability should be based on those batches with the higher moisture content. Such data should also be considered in the establishment of a moisture specification.

For products showing a loss of potency due to aging, there are generally two potency specifications. There is a higher limit for the dosage form at the time of release. This limit is generally higher than the official USP or filed specification which is official throughout the entire expiration date period of the dosage form. The USP points out that compendial standards apply at any time in the life of the article.

Stability testing should also include provisions for the assay of aged samples and subsequent reconstitution of these aged samples for the maximum amount of time specified in the labeling. On some occasions, manufacturers have established expiration dates without performing label claim reconstitution potency assays at the various test intervals and particularly the expiration date test interval. Additionally, this stability testing of reconstituted solutions should include the most concentrated and the least concentrated reconstituted solutions. The most concentrated reconstituted solution will usually exhibit degradation at a faster rate than less concentrated solutions.

(c) Sterility Testing

With respect to sterility testing of lyophilized products, there is concern with the solution used to reconstitute the lyophilized product. Although products may be labeled for reconstitution with Bacteriostatic Water For Injection, Sterile Water For Injection (WFI) should be used to reconstitute products. Because of the potential toxicities associated with Bacteriostatic Water For Injection, many hospitals only utilize WFI. Bacteriostatic Water For Injection may kill some of the vegetative cells if present as contaminants, and thus mask the true level of contamination in the dosage form.

As with other sterile products, sterility test results which show contamination on the initial test should be identified and reviewed.

FINISHED PRODUCT INSPECTION - MELTBACK

The USP points out that it is good pharmaceutical practice to perform 100% inspection of parenteral products. This includes sterile lyophilized powders. Critical aspects would include the presence of correct volume of cake and the cake appearance. With regard to cake appearance, one of the major concerns is meltback.

Meltback is a form of cake collapse and is caused by the change from the solid to liquid state. That is, there is incomplete sublimation (change from the solid to vapor state) in the vial. Associated with this problem is a change in the physical form of the drug substance and/or a pocket of moisture. These may result in greater instability and increased product degradation.

Another problem may be poor solubility. Increased time for reconstitution at the user stage may result in partial loss of potency if the drug is not completely dissolved, since it is common to use in-line filters during administration to the patient.

Manufacturers should be aware of the stability of lyophilized products which exhibit partial or complete meltback. Literature shows that for some products, such as the cephalosporins, that the crystalline form is more stable than the amorphous form of lyophilized product. The amorphous form may exist in the "meltback" portion of the cake where there is incomplete sublimation.

GLOSSARY

ATMOSPHERE, THE EARTH

The envelope of gases surrounding the earth, exerting under gravity a pressure at the earth's surface, which includes by volume 78% nitrogen, 21% oxygen, small quantities of hydrogen, carbon dioxide, noble gases, water vapor, pollutants and dust.

ATMOSPHERIC PRESSURE

The pressure exerted at the earth's surface by the atmosphere. For reference purposes a standard atmosphere is defined as 760 torr or millimeters of mercury, or 760,000 microns.

BACKSTREAMING

A process that occurs at low chamber pressures where hydrocarbon vapors from the vacuum system can enter the product chamber.

BLANK-OFF PRESSURE

This is the ultimate pressure the pump or system can attain.

BLOWER (see Mechanical Booster Pump)

This pump is positioned between the mechanical pump and the chamber. It operates by means of two lobes turning at a high rate of speed. It is used to reduce the chamber pressure to less than 20 microns.

BREAKING VACUUM

Admitting air or a selected gas to an evacuated chamber, while isolated from a vacuum pump, to raise the pressure towards, or up to, atmospheric.

CIRCULATION PUMP

A pump for conveying the heat transfer fluid.

CONDENSER (Cold trap)

In terms of the lyophilization process, this is the vessel that collects the moisture on plates and holds it in the frozen state. Protects the vacuum pump from water vapor contaminating the vacuum pump oil.

CONDENSER/RECEIVER

In terms of refrigeration, this unit condenses (changes) the hot refrigerant gas into a liquid and stores it under pressure to be reused by the system.

COOLING

The lowering of the temperature in any part of the temperature scale.

CONAX CONNECTION

A device to pass thermocouple wires through and maintain a vacuum tight vessel.

CONTAMINATION

ln the vacuum system, the introduction of water vapor into the oil in the vacuum pump, which then causes the pump to lose its ability to attain its ultimate pressure.

DEFROSTING

The removal of ice from a condenser by melting or mechanical means.

DEGREE OF CRYSTALLIZATION

The ratio of the energy released during the freezing of a solution to that of an equal volume of water.

DEGREE OF SUPERCOOLING

The number of degrees below the equilibrium freezing temperature where ice first starts to form.

DESICCANT

A drying agent.

DRY

Free from liquid, and/or moisture.

DRYING

The removal of moisture and other liquids by evaporation.

EQUILIBRIUM FREEZING TEMPERATURES

The temperature where ice will form in the absence of supercooling.

EUTECTIC TEMPERATURE

A point of a phase diagram where all phases are present and the temperature and composition of the liquid phase cannot be altered without one of the phases disappearing.

EXPANSION TANK

This tank is located in the circulation system and is used as a holding and expansion tank for the transfer liquid.

FILTER OR FILTER/DRIER

There are two systems that have their systems filtered or filter/dried. They are the circulation and refrigeration systems. In the newer dryers this filter or filter/dryer is the same, and can be replaced with a new core.

FREE WATER

The free water in a product is that water that is absorbed on the surfaces of the product and must be removed to limit further biological and chemical reactions.

FREEZING

This is the absence of heat. A controlled change of the product temperature as a function of time, during the freezing process, so as to ensure a completely frozen form.

GAS BALLAST

Used in the vacuum system on the vacuum pump to decontaminate small amounts of moisture in the vacuum pump oil.

GAS BLEED (Vacuum control)

To control the pressure in the chamber during the cycle to help the drying process. In freeze-drying the purpose is to improve heat-transfer to the product.

HEAT EXCHANGER

This exchanger is located in the circulation and refrigeration systems and transfers the heat from the circulation system to the refrigeration system.

HEAT TRANSFER FLUID

A liquid of suitable vapor pressure and viscosity range for transferring heat to or from a component, for example, a shelf or condenser in a freeze-dryer. The choice of such a fluid may depend on safety considerations. Diathermic fluid.

HOT GAS BYPASS

This is a refrigeration system. To control the suction pressure of the BIG FOUR (20-30 Hp) compressors during the refrigeration operation.

HOT GAS DEFROST

This is a refrigeration system. To defrost the condenser plates after the lyophilization cycle is complete.

ICE

The solid, crystalline form of water.

INERT GAS

Any gas of a group including helium, radon and nitrogen, formerly considered chemically inactive.

INTERSTAGE

In a two stage compressor system, this is the cross over piping on top of the compressor that connects the low side to the high side. One could also think of it as low side, intermediate, and high side.

INTERSTAGE PRESSURE REGULATING VALVE

This valve controls the interstage pressure from exceeding 80 - 90 PSI. This valve opens to suction as the interstage pressure rises above 80 - 90 PSI.

LEXSOL

A heat transfer fluid (high grade kerosene).

LIQUID SUB-COOLER HEAT EXCHANGER (see Sub-cooled Liquid)

The liquid refrigerant leaving the condenser/receiver at cooling water temperature is sub-cooled to a temperature of +150F (-100C) to -150F (-250C).

LYOPHILIZATION

A process in which the product is first frozen and then, while still in the frozen state, the major portion of the water and solvent system is reduced by sublimation and desorption so as to limit biological and chemical reactions at the designated storage temperature,

MAIN VACUUM VALVE (see Vapor Valve)

This valve is between the chamber and external condenser to isolate the two vessels after the process is finished. This is the valve that protects the finished product.

MATRIX

A matrix, in terms of the lyophilization process, is a system of ice crystals and solids that is distributed throughout the product.

MECHANICAL BOOSTER PUMP (see Blower)

A roots pump with a high displacement for its size but a low compression ratio. When backed by an oil-seal rotary pump the combination is an economical alternative to a two-stage oil-sealed rotary pump, with the advantage of obtaining a high vacuum.

MECHANICAL VACUUM PUMP

The mechanical pumping system that lowers the pressure in the chamber to below atmospheric pressure so that sublimation can occur.

MELTING TEMPERATURE (Melt-back)

That temperature where mobile water first becomes evident in a frozen system.

MICRON (see Torr)

A unit of pressure used in the lyophilization process. One micron = one Mtorr or 25,400 microns = 1" Hg., or 760,000 microns = one atmosphere.

NONCONDENSABLES

A mixture of gases such as nitrogen, hydrogen, chlorine, and hydrocarbons. They may be drawn into the system through leaks when part of the system is under a vacuum. Their presence reduces the operating efficiency of the system by increasing the condensing pressure.

NUCLEATION

The formation of ice crystals on foreign surfaces or as a result of the growth of water clusters.

OIL-MIST FILTER

In vacuum terminology a filter attached to the discharge (exhaust) of an oil-sealed rotary pump to eliminate most of the "smoke" of suspended fine droplets of oil which would be discharged into the environment.

OIL SEALED ROTARY PUMP

A standard type of mechanical vacuum pump used in freeze-drying with a high compression ratio but having a relatively low displacement (speed) for its size. A two-stage pump is effectively two such pumps in series and can obtain an ultimate vacuum.

OIL SEPARATOR

Separates the oil from the compressor discharge gas and returns the oil through the oil float trap and piping to the compressor crankcase.

REAL LEAK

A real leak is a source of atmospheric gases resulting from a penetration through the chamber.

RECONSTITUTE

The dissolving of the dried product into a solvent or diluent.

RELIEF VALVE

Used for safety purposes to prevent damage in case excessive pressure is encountered.

ROTARY VANE PUMP

A mechanical pumping system with sliding vanes as the mechanical seal. Can be single or two stages.

SHELF COMPRESSOR (Controlling Compressor)

Used for controlling the shelf temperature, either cooling or from overheating.

SELF LIQUID HEAT EXCHANGER

The transfer of heat from the shelf fluid to the refrigeration system through tubes in the exchanger causing compressor suction gas to warm.

SHELVES

In terms of the lyophilization process, they are a form of heat exchanger, within the chamber, that have a serpentine liquid flow through them, entering one side and flowing to the other side. They are located in the circulation system.

SINGLE STAGE COMPRESSOR

This is a normal type compressor used in refrigeration. In the lyophilization process it is used to control the shelf temperature, both for cooling and keeping the shelf temperature from overheating using a temperature controller.

SILICONE OIL

A heat transfer fluid.

STERILIZATION

The use of steam and pressure to kill any bacteria that may be able to contaminate that environment or vessel.

SUBLIMATION

The conversion of a material from a solid phase directly to a vapor phase, without passing through the liquid phase. This is referred to as the primary drying stage.

SUB-COOLED LIQUID (See Liquid Sub-cooler Heat Exchanger)

The liquid refrigerant is cooled through an exchanger so that it increases the refrigerating effect as well as reduces the volume of gas flashed from the liquid refrigerant in passage through the expansion valve.

SUCTION LINE ACCUMULATOR

To provide adequate refrigerant liquid slug protection (droplets of liquid refrigerant) from returning to the compressor, and causing damage to the compressor.

TCE

Trichloroethylene - A heat transfer fluid.

TEMPERATURE

The degree of hotness or coldness of a body.

THERMOCOUPLE

A metal-to-metal contact between two dissimilar metals that produces a small voltage across the free ends of the wire.

THERMOSTATIC EXPANSION VALVE

An automatic variable device controlling the flow of liquid refrigerant.

TORR (See Micron)

A unit of measure equivalent to the amount of pressure in 1000 microns.

TWO STAGE COMPRESSOR (see Interstage)

This is a specially built compressor. Its function is to be able to attain low temperatures by being able to operate at low pressures. It is two compressors built into one. A low stage connected internally and a high stage connected externally with piping, called interstage.

UNLOADING VALVE

This valve connects the interstage with suction to equalize both pressures during pump-down.

VACUUM

Strictly speaking, a space in which the total pressure is less than atmospheric.

VACUUM CONTROL (Gas Bleed)

To assist in the rate of sublimation, by controlling the pressure in the lyophilizer.

VACUUM PUMP

A mechanical way of reducing the pressure in a vessel below atmospheric pressure to where sublimation can occur. There are three types of pumps, rotary vane, rotary piston and mechanical booster.

VAPOR BAFFLE

A target shaped object placed in the condenser to direct vapor flow and to promote an even distribution of condensate.

VACUUM VALVES

The vacuum valves used are of a ball or disk type that can seal without leaking. The ball types are used for services to the chamber and condenser. They are also used for drains and isolation applications. The disk types are used in the vacuum line system and are connected to the vacuum pump, chamber and condenser.

VAPOR VALVE (See Main Vacuum Valve)

The vacuum valve between the chamber and external condenser. When this valve is closed the chamber is isolated from the external condenser. Also known as the main vapor valve.

VIAL

A small glass bottle with a flat bottom, short neck and flat flange designed for stoppering.

VIRTUAL LEAK

In the vacuum system a virtual leak is the passage of gas into the chamber from a source that is located internally in the chamber.

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REVIEW PAPER

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LYOPHILIZATION – THE PROCESS AND INDUSTRIAL USE

This article presents a general overview of lyophilization and discusses the underlying principles of the process through the basics of: formulation, freezing, primary drying and secondary drying. In this article lyophilization is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by desorption (secondary drying) to values that will no longer support biological growth or chemical reactions. Special mention was made of the industrial use of the process and emphasis was placed on the lyophilization of pharmaceutical products and food industry products. Lyophilization equipment, as well as the formulation of materials that can be lyophilized, are described in sufficient detail to give information on the restrictions and advantages of lyophilization. Processing economics and comparison with conventional drying methods are presented. A historical overview of the process and future developments presented from the industrial viewpoint give an insight on the previous application of lyophilization and the prospects of its broad industrial use.

INTRODUCTION TO FREEZE DRYING TECHNOLOGY

Historical Overview

Historically, lyophilization can be traced back to prehistory when, for example, the Eskimo preserved fish by dehydration in the cold, dry Arctic winds.

The history of lyophilization as an industrial process is surprisingly recent. Although Altmann [1] used lyophilization for the preparation of histological sections as early as 1890, his technique went unnoticed for over 40 years. Shackell [2] independently rediscovered the technique in 1909 for the preservation of biologicals.

The industrial applications of lyophilization do not appear to have been appreciated until when there arose the need to process large quantities of heat-sensitive blood products and the recently discovered antibiotics. The efforts of Tival in 1927 and Elser in 1934 were rapidly followed by the important contributions of Flosdorf in the United States and Greaves in England who were largely responsible for making large scale applications of lyophilization possible. Stimulated particularly by a series of symposia in England and the United States and the renowned courses on lyophilization organized by Rey in France, the atmosphere in the 1950s and early 60s was one of optimism for the future of lyophilization, particularly in its application to foodstuffs [3].

Three serious practical disadvantages of freeze preservations:

1. the high cost of transporting these stocks of frozen product,
2. the associated high cost of transporting these stocks and
3. the potential for total loss of material due to failure of the freezing plant,

have encouraged the adoption of drying methods. Conventional drying techniques, using high process temperatures, while successful in dehydrating material often result in alterations of the organoleptic properties, a reduction in nutritional value or a decrease in biological activity in the case of an enzyme, hormone etc. Lyophilization is a well established alternative technique used to preserve labile materials often, but not exclusively, of biological origin [4–6].

With advances in vacuum and refrigeration technologies reliable commercial freeze-dryers were able to be developed [7]. Since that time the method has become fully integrated within the pharmaceutical industry, and is particularly useful for establishing an early lead when marketing a novel therapeutic agent.

Definitions

There are two terms used in industry which describe the same process but in a different way: Freeze drying and lyophilization, which means 'to make solvent-loving'.

Rey [8] first came up with the term lyophilization by taking into account the porous nature of the dried product and its "lyophil" characteristic to rapidly reabsorb the solvent and restore the substance. The term lyophilization has become more common because it is applicable to both aqueous and non-aqueous systems. It is interesting that lyophilization processes are often conducted in lyophilization equipment, although the descriptive term lyophilizer is becoming more prevalent. In this article, the process will be defined as lyophilization, and the equipment will still be referred to as a freeze-dryer.

Although the steps in the lyophilization process outlined by Rey [8] have been generally accepted, only recently has a definition been given to the term, lyophilization [9]. In its simplest form, lyophilization is defined as a stabilizing process in which the substance is first frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then

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by desorption (secondary drying) to values that will no longer support biological growth or chemical reactions [10].

The advantages of Lyophilization

Apart from the reduction in thermal inactivation of the product during freezing as noted above, other advantages of the method are:

- The avoidance of concentration effects – for example salting out of proteins. The distribution of components within the drying and dried product remain unchanged throughout the process.
- The water content of the dried product can be reduced to very low levels. In general the lower the water content the more stable the product, although overdrying may reduce product stability.
- Since the product is normally sealed under vacuum or inert gas, oxidative denaturation is greatly reduced.
- Loss of water equates to a loss of product weight and this may be important where transport costs are significant.
- Lyophilization operates in a more controlled environment than some other forms of drying, which potentially means less possibility for contamination.

Because freeze material has a porous structure, the product can be instantly reconstituted by the addition of a simple diluent [11].

Materials That Can Be Lyophilized

The major types of material processed by lyophilization have been summarized by Adams [12]:

1. Non–biological where the process is used to dehydrate or concentrate reactive or heat labile chemicals

2. Non–living bioproducts – this comprises the major area of application and includes:

- Enzymes, hormones, antibiotics, vitamins, blood products, antibodies, inactivated vaccines, etc. This sub–group includes pharmaceuticals which may be used diagnostically or therapeutically
- Bone and other body tissues for surgical or medical use
- Foodstuffs where organoleptic properties are important
- Industrially useful bio products

3. Living organisms – where reconstituted cells after drying must be able to grow and multiply to produce new progeny. Examples include bacteria and fungi used as seed cultures or attenuated viral vaccines.

4. Miscellaneous – Flood damaged books, museum artifacts, etc.

Lyophilization is less suitable for materials which supercool to form glasses, for products which form impervious surface skins upon cooling, thereby inhibiting the evolution of subliming vapour, and is unsuitable for certain cell types (eukaryotes) which are able to retain viability, when frozen, only in the presence of special additives incompatible with the lyophilization.

THE PROCESS

The following is a general overview of the lyophilization process. The intent of this section is merely to familiarize the reader with the basic steps in the process.

Formulation

A formulation is defined as any system containing a solvent that, upon its removal, will enhance the stability of the substance.

Formulation preparation is often neglected when discussing lyophilization. It is important to remember that lyophilization is invariably the ultimate step in a preparation exercise and one should realise that lyophilization will never rectify damage caused during preparation but will rather exacerbate any such damage. Foodstuffs should be of good quality and may require special pretreatment. For example, muscle tissue should be sliced in such a way as to minimise the impedance to vapour flow offered by skins and membranes, while vegetables may require blanching to prevent oxidative browning. Solutions intended for pharmaceutical use generally consist of an active constituent and possibly other constituents such as protective additives that are added to stabilize the formulation in its liquid state for therapeutic reasons. Pharmaceutical formulations will require dispensing into suitable containers prior to freezing [13].

Containers must be regarded as an integral part of the processing equipment since their nature and design will influence heat transfer and impede vapour flow to a greater or lesser extent.

Foodstuffs, chemicals, bulk pharmaceuticals, etc may be dispensed into aluminium, teflon coated or stainless steel trays. While metals have superior thermal conductive properties compared to plastics, thin walled plastic trays are nevertheless becoming increasing by popular for processing bulk materials.

Pharmaceutical solutions are usually dispensed into glass vials or ampoules. Vials are small bottles fitted with a special ventilated stopper which permits the escape of water vapour during drying (Figure 2). At the end of the process the stoppers can be fully inserted into the vial using the stoppering device within the freeze–drier (See Section **Container–Closure System**) to produce a completely sealed container. While vials are particularly convenient for filling and lyophilization, the vial stopper hybrid has the potential to leak between the vial neck and stopper [14]. The all–glass ampoule theoretically overcomes the potential for leakage since the ampoule tip is heat fused at the end of drying. Practical difficulties associated with heat sealing ampoules within the freeze–drier require that the ampoules are sealed externally and complicated techniques must be adopted to prevent the ingress of damp air into the ampoule during sealing. Cammack and Adams [15] recommended that vials are used for products requiring only short term storage while

ampoules are more satisfactory where long term storage is essential. One should be alert to the possibility that poor heat-sealing can cause ampoule leakage during storage [16].

Ampoules and vials should be manufactured from good quality glass and ideally should be fabricated from glass tubing. The walls and bases should be oven and stress-free to enable a consistent product freezing and drying pattern to be obtained with minimum breakages due to thermal shock during prefreezing. Light sensitive products may require dispensing into amber or coloured vials prior to processing.

Rubber stoppers should be manufactured from ethically acceptable polymers and are usually lubricated with silicone oil as an aid to insertion. Because of the potential for silicone fluid to cause product denaturation, stoppers coated with alternative lubricants (such as teflon) have recently been introduced. The washing and pretreatment of stoppers should be carefully controlled prior to filling, since it is possible for stoppers to absorb significant quantities of water during washing and sterilization. Sufficient water may remain within the stopper even after lyophilization to cause product collapse and reduced stability upon storage. Product collapse is the loss of structure or the noticeable shrinkage of product observed in a vial during or following lyophilization. In extreme cases collapse will result in the formation of an amorphous, bubbled, glassy residue within the vial. Collapse may be confused with melting, although the two phenomena are quite distinct.

Recently, novel containers have appeared for specialist use. Sealed containers, holding both a plug of dried product and a quantity of diluent can now be processed enabling the clinician to reconstitute the freeze-dried material with simplicity. Such containers minimise the risk of product contamination or the mismatching of drug with diluent during reconstitution. Small beads and plastic wells holding type - specific sera, adhesive plasters impregnated with wound-healing freeze-dried hormones, nasal inhalers containing freeze-dried vaccines or anti-congestants and instantly dissolving tablets are among the range of novel containers and techniques being used for the processing and distributing freeze-dried products.

Lyophylization in glass containers will serve as the basis for an overview of the process, as the intention is to emphasis the use of the process in the pharmaceutical industry, where lyophylization in glass containers prevails [17].

Freezing

The principal function of the freezing process is:

- to separate the solvent from the solutes.
- to minimise thermal degradation within the dispersed product.
- to immobilise the components in the solution thereby preventing their concentration during drying.

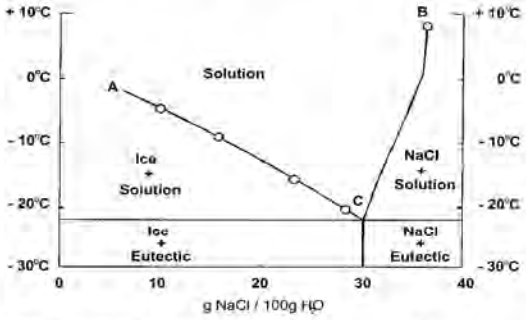


Figure 1. The eutectic phase diagram of sodium chloride solution [18]

- to prevent product foaming when vacuum is applied.

For an aqueous system, the water will form ice crystals and solutes will be confined to the interstitial region between the ice crystals. The temperature necessary to achieve complete freezing of the formulation will depend on the nature of the solvent and other constituents that comprise the formulation. Freezing may be performed in an external freezing unit or on the shelves of the freeze-dryer.

An example of the frozen ice-product matrix is given in Figure 2b. The freezing process is an important step in the lyophilization process.

Ideally prefreezing should result in the complete crystallisation of both the solvent (usually) water and the solutes within the solution. When discussing the formulation, we shall see that this idea is seldom achieved in practice. Figure 1 describes the eutectic phase diagram of an aqueous sodium chloride solution which could be translated into a description of events occurring during prefreezing. (The eutectic point is the temperature in a system at which a residual liquid phase is in equilibrium with the solid phases (Figure 1). In this case, cooling from room temperature to 0°C is followed by supercooling prior to the crystallisation of pure water as ice, thereby resulting in a concentration of the sodium chloride solution. Reducing the temperature still further will increase the concentration of the salt solution during the eutectic transition. A second supercooling inflexion is followed by the crystallisation of the remainder, of the water and the salt at the eutectic point, resulting in a mixture of ice and salt crystals.

Rates of Freezing Prior to Sublimation

When considering the cooling rates obtained on the shelves of the freeze-dryer, one must appreciate that cooling proceeds from the base of the vial upwards through the depth of the liquid. In this case it is useful to regard freezing rates as depths of liquid frozen, in millimeters per minute. The optimal rate of cooling prior to sublimation is 1mm/min and such a freezing rate will

induce the correct ice crystal formation necessary to permit water vapour to escape freely from the frozen matrix. At freezing rates below 0.5 mm or much greater than 1.0 mm per minute, the structure of the ice matrix will be such that vapour flow will be impeded during sublimation and drying times significantly extended. It is not possible to achieve this optimal cooling rate when the depth of the fill in the vial greatly exceeds 10 mm and this depth should therefore be regarded as the maximum fill depth. Liquid foodstuffs, such as coffee, are often spray or droplet frozen prior to lyophilization.

Problems Associated With Incomplete Freezing

Incomplete freezing may result as a consequence of mechanical failure of the shelf or cabinet cooling system or a failure to allow an adequate prefreezing time to ensure complete product freezing. Incomplete freezing can also result from an inability to cool the product below an eutectic temperature. Suspensions of micro-organisms often contain several electrolytes and should be cooled below the lowest eutectic temperature to ensure complete freezing. It is imperative that a vacuum is not applied to an incompletely frozen product since foaming may occur resulting in an unacceptable product.

Primary Drying

Once the formulation has reached a completely frozen state, the pressure in the freeze-dryer is reduced,

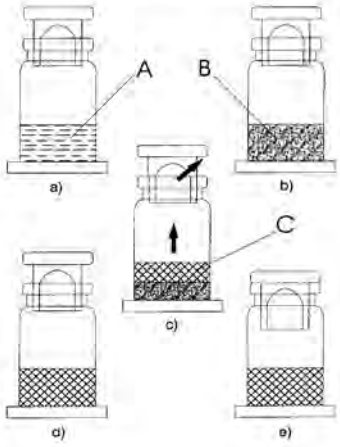


Figure 2. Lyophilization in glass containers [10]
Fig. 2a) shows a fill volume of a liquid formulation, denoted by the region defined as "A" in a glass container with a lyophilization closure positioned for the drying process; in Fig. 2b) The frozen ice-product matrix of the formulation is designated by the region "B"; Fig. 2c) illustrates the primary drying process and the interstitial cake portion is denoted as region "C"; the completion of secondary drying is shown by Fig. 2d), and the final product with the closure in its stoppered position is illustrated by Fig. 2e).

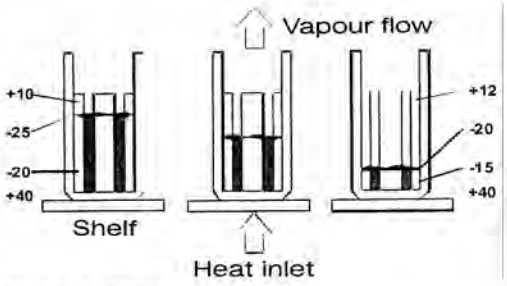


Figure 3. Sublimation

and heat is applied to the formulation to initiate sublimation of the ice crystals. The application of vacuum allows the free migration of water vapour from the frozen mass. The migration of water from the product may be regarded basically as a diffusion process and this will help to appreciate why lyophilization is a relatively slow process. The sublimating solvent vapors pass through the opening in the closure. As the sublimation of the ice crystals proceeds, the ice-gas interface recedes through the cake (Figure 2c). As there are latent heat losses during sublimation, heat must be applied to the product throughout primary drying. In the absence of heat, the product temperature would reach a level at which lyophilization would cease. It is important that heat is not applied until a vacuum of c.20 Pa has been attained or product melting may occur. In the case of pharmaceutical application of the process the vials are placed on shelves which facilitates heat exchange as shown in Figure 3.

Throughout most of the primary drying cycle the product will remain at a significantly lower temperature than the shelf because of heat loss with water vapour escape from the product and only toward the end of primary drying, when sublimation has virtually ceased, will the product temperature rapidly rise to that of the shelf (Figure 4). Completion of the primary drying process occurs when all of the ice crystals have been removed from the formulation and the volume occupied by the resulting cake is equivalent to that of the frozen

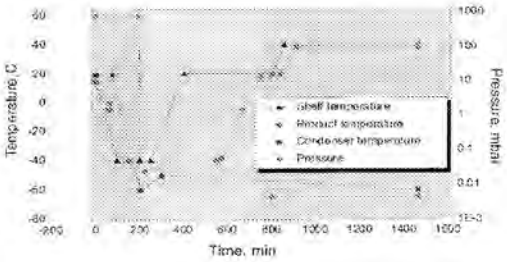


Figure 4. Idealised lyophilisation cycle

matrix. Only water adsorbed to the bulk powder or structurally integrated with the solute will remain. The product could be removed from the drier at this stage. In practice, since the water content is usually too high, 5–10% w/w for optimal stability, the drying cycle is extended.

Secondary Drying

After the completion of primary drying (Figure 1.1d), there will still be some water adsorbed onto the surface of the cake. This moisture may constitute, depending on the temperature and the nature of the constituents comprising the cake, 5–10% w/w of the dried product. In many cases, such moisture values may be too high, and the final product may not have the desired stability. The desired stability is obtained by reducing the moisture content in the product by desorbing the moisture from the cake without reducing the volume of the interstitial cake. This prolonged drying stage is called secondary drying, water being removed by desorption. The remaining water removal is usually accomplished by increasing the temperature of the product and reducing the partial pressure of water vapor in the container. During secondary drying the water content is reduced to 2% or less and various methods to determine the moisture content are used [19].

Sensitive biological materials may be inactivated by overdrying [20]. In older accounts of lyophilization methodology, it was often recommended that primary and secondary drying should be completed using separate vapour traps within the freeze-drier or even completed on separate machines. The greater efficiency of vapour trapping systems in modern plants no longer require special secondary drying devices.

Container–Closure System

After lyophiilization, the formulation must be protected from the environment. In most cases, the formulation in the container is sealed by a stoppering mechanism contained in the freeze-dryer, which depresses closure into the container (Figure 2e). The stoppering of the closure into the container temporarily protects the final product from the environment. Upon completion of the stoppering of the containers, the product can be safely removed from the freeze-dryer and the stopper crimp-sealed with a metal or colored cap to provide a permanent seal for the product.

THE BASIC DESIGN OF FREEZE–DRYERS

The following is a brief, general description of the essential components and their functions in a freeze-dryer. The general layout of a freeze-dryer is given in Figure 5.

The Lyophilization Chamber

The freeze-dryer chamber serves two main functions: to provide a safe environment for the product during the entire lyophilization process and to provide

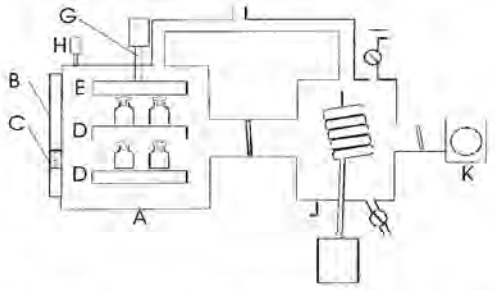


Figure 5. The general layout of a freeze–dryer
A is the drying chamber; B is the door to the drying chamber and C is a viewing port for a metal door. D represents the usable shelves of the dryer, while E is an unusable shelf. G represents the hydraulic ram system. H is the pressure gauge, I is the condenser surface housed in an insulated condenser chamber J, and K represents the vacuum pumping system.

the necessary temperatures and pressures to conduct each step of the lyophilization process.

The lyophilization chamber (A), as illustrated by Figure 5, is a metal vessel, generally constructed from stainless steel that can be accessed by a hinged door (B). The chamber door is either fabricated from metal such as stainless steel or from clear plastic. Figure 5 shows that the chamber is accessed by means of a metal door containing a glass viewing port (C). The door is fitted with an elastomer gasket (not shown) to form a vacuum seal with the drying chamber.

The drying chamber also contains a pressure gauge (H) and is equipped with an insulation covering over the entire chamber surface to prevent heat transfer to the shelves (D) and the trays (F) during the drying process.

Shelves

A small research freeze-dryer may have only one shelf but all others will have several. The shelf, design is made more complicated because of the several functions it must perform. The shelf acts as a heat exchanger, removing energy from the product during freezing, and supplying energy to the product during the primary and secondary drying segments of the freeze drying cycle. Shelves must be flat and level to ensure there is proper contact with the product containers. The shelf construction must be heavy enough to ensure that a level surface is maintained and strong enough to support the forces of a stoppering system if fitted. All of the shelves are of a hollow construction that permits the serpentine flow of the heat-transfer fluid. The heat-transfer fluid can be chilled to freeze the product or heated to provide the necessary energy for the primary and secondary drying processes. A shelf stack may be fixed in to position where bulk products are handled or a hydraulic system (G) can move the shelves vertically to provide the necessary force to stopper the closures prior to opening the chamber door (B).

The shelves are connected to a silicone oil system through either fixed or flexible hoses.

The drying chamber presented in Figure 5 contains usable shelves (D) and an unusable shelf (F). The trays (F) containing the product, either bulk or in glass containers, are loaded onto the usable shelves. The unusable shell (E) serves as a radiation shield for the top shelf.

The Condenser Chamber

The main function of the condenser chamber is to house the condenser surfaces for the removal of water vapor from the gases that pass from the drying chamber. For the condenser plates to be effective, their operating temperatures must be a minimum of 20°C lower than the product temperature during the primary drying process. Unlike the shelves of the dryer, which are chilled by a heat-transfer fluid, the condenser surfaces are generally refrigerated by the direct expansion of a refrigerant.

Figure 5 illustrates an external condenser system in which the condenser surfaces (I) are housed in a separate insulated vacuum chamber (J). In some dryers, the condenser surfaces are housed in the drying chamber and are referred to as internal condensers.

The Vacuum Pumping System

The vacuum pumping system, in conjunction with the condenser system, provides the necessary pressures for conducting the primary and secondary drying processes. Typically mechanical vacuum pumps used in freeze-dryers are oil lubricated, however, oil-free mechanical pumping systems are available. The vacuum pump (K) shown in Figure 5 compresses the non-condensable gases that pass through the condenser chamber (J) and discharges these gases directly into the atmosphere.

Sensors

The temperature and pressure must be measured during the process [21]. The two parameters which are controlled are the fluid or shelf temperature and the pressure (or vacuum). Other temperatures which can be measured (during a lyophilization cycle) include product temperatures and the process condenser. The temperature measuring devices used are RTDs (PT 100) or thermocouples (normally Type T). Vacuum sensors include two main types - thermoelectric or Pirani gauges and capacitance manometers.

Control System

Control may be entirely manual or usually fully automatic for the production machines. The control elements required are as mentioned above, shelf temperature and pressure plus time. A control programme will set up these values as required by the product or the process. The time may vary from a few hours to several days. Other data such as product temperatures and process condenser temperatures can also be recorded and logged [22,23].

APPLICATIONS

The following is a general discussion of the various applications of the lyophilization process in the various industries. The intent of the discussion is not to provide the reader with a comprehensive review, but with an overview of the scope of the lyophilization process.

Healthcare Industry

The most extensive use of the lyophilization process is involved in the healthcare industry. This includes the lyophilization of pharmaceuticals such as chemical compounds, parenteral formulations, vaccines, and also in diagnostic products. Included in this category are biotechnology products that consist mainly of protein-based products [24,25]. A listing of pharmaceutical products given in Table 1 below is provided by courtesy of the Hemofarm Group and serves as an example of the variety of materials that are lyophilized for use in healthcare (Table 1).

Table 1. Materials that are lyophilized for use in healthcare

Product	Active Ingridient	Pharmacological Group
Lemod Solu®	Methylprednisolone	Corticosteroid
Hidrokortizon® (Hemol®)	Hydrocortisone	Corticosteroid
Azaran®	Ceftriaxone	Antibiotic
Fenobarbiton-Na®	Phenobarbitone	Antiepileptic
Nitroprusid-Na®	Nitroprusside	Vasodilator

Veterinary

Perhaps the second most common use of lyophilization involves veterinary products. These products range from vaccines for individual household pets to large scale applications, such as the inoculation of herds of cattle and sheep or large flocks of poultry. These lyophilized products serve not only to protect the animals and the investment of the producer, but also to improve the quality of the product. These products also protect the consumer from diseases that may be transmitted by the consumption of animal products.

Food

The most widely known freeze-dried food product is coffee. However, freeze-dried coffee is not, in the true sense, a lyophilized product. Its dark brown color stems not from the coffee solution but from the manner in which the product is prepared. The coffee is first extracted from the coffee bean with hot water and the water content is reduced to form a concentrated viscous extract. The extract is then carefully processed to form frozen granules. These coffee granules are then dried

under conditions that generate partial collapse or controlled meltback [26], which provide the granules with the dark brown color. If the coffee were truly lyophilized, the color would appear golden brown because of the reflection of light from the surfaces of the frozen coffee solids. In addition, lyophilzed coffee would require a cup of lyophilized powder for each cup of coffee instead of the single teaspoon that is currently used. As a result of the application of the term freeze-dried to a form of instant coffee, lyophilization is no longer synonymous with lyophilization.

Other freeze food products are manufactured. These products may be used as additives, such as berries in breakfast cereals, or are complete products that eliminate the need for refrigeration, such as ice cream that can be eaten without reconstitution. The lyophilization of foods is also applicable for products where weight can be a factor, such as in backpacking. These freeze-dried meals, like freeze-dried beef, require reconstitution by the addition of water and enhancement of flavor by heating the product [27]. Except in special cases like freeze-dried coffee, freeze-dried foods cannot compete economically with frozen or canned food products.

Other Applications

Other applications of lyophilization include the lyophilization of floral products and taxidermy. There has only been limited success in the lyophilization of floral products; only the tile flower portion of the plant appears to respond to the drying process, and there is often a slight change in color. For example, red roses tend to take on a purple tint. The stems of the flowers tend to assume a brown tint, and the leaves become brittle and curled. In the drying of plants, the nature of the plant plays an important role. For example, cacti, in order to conserve water, are equipped with an outer layer that is nearly impervious to the transport of water vapor. A plant such as a cacti would prove difficult or nearly impossible to dry without first disturbing the outer layer to permit the transport of water vapor. While the preservation of flowers by lyophilization techniques could represent a major market, the growth of the market awaits the development of a better process. Pretreatment of the flower prior to lyophilization, when freshly cut or even during its growth, may be necessary in order to achieve a more natural product with a relatively long shelf life.

Lyophilization has been applied to the taxidermy of small animals. Many small mammals, such as squirrels and raccoons, have been successfully freeze-dried to provide a specimen having a lifelike appearance. In spite of the success of this technique, it is currently used on a very limited basis.

Lyophilization has been instrumental in the rescue of precious manuscripts, books, and documents that incurred water damage as a result of a fire or a flood. By quickly freezing all of the documents and then lyophilizing the documents in batches, many documents and valuable manuscripts have been restored to nearly original condition. While such services are not often required, the fact that this technology has applications that can prove beneficial to later generations is indeed a rewarding legacy provided by this technology.

PROCESSING ECONOMICS

It is difficult to compare the economics of lyophilization accurately with conventional drying partly because of the problems of conducting trials using identical materials processed under similar conditions of scale and partly because the results of realistic trials would presumably be commercially sensitive.

Processing Time

Table 2 illustrates the comparative drying times for typical products spray, fluid bed or freeze-dried. The considerably increased processing time for lyophilization should be noted. Product capacity is further reduced since the depth of fill is insured to 1 cm for technical reasons.

Table 2. Temperatures and drying times for drying processes

Process		Typical Operating Temperatures	Drying Times
Spray Drying		80–100°C	< 10 min
Fluid Bed Drying		80°C	< 100 min
Freeze drying (1 cm product depth)	Milk	- 5°C	600 min
	Blood Serum	- 25°C	1200 min
	Cytomegalovirus Vaccine	- 40°C	3600 min

Equipment Capital Costs

Modern pharmaceutical freeze-driers are an amalgam of a refrigeration/vacuum/steam pressure plant and, consequently, equipment design and fabrication costs are high.

Operating Costs

In addition to the normal running costs involved with operating an industrial plant, the lyophilization process depends on the extraction or addition of energy to freeze, sublime and condense water vapour. This additional removal of latent heat increases the cycle of energy consumption considerably [28]. For a significant period of the cycle time during freezing, pharmaceutical freeze-driers are utilized only as a freezing cabinet thereby further reducing the economic efficiency of the plant. Incidental factors which increase production costs include the requirement to use high cost dosage containers and the additional costs of sealing the dried product.

Comparative Economics

From the limited data available, a cost comparison of processing foodstuffs would indicate a 5–8 fold

increase in drying costs between lyophilization and conventional drying [29]. However, the point should be made that when the entire process is compared, that is from raw material to finished product, this differential may be significantly reduced in favour of freeze drying and a finished product cost comparison of conventional drying: lyophilization of 1:1.33 may be feasible [30].

On purely economic grounds lyophilization is likely to compare unfavourably with alternative drying techniques and the process can only be justified on the basis of superior product quality.

PHARMACEUTICAL USE OF THE LIOPHILIZATION PROCESS

The lyophilization process generally includes the following steps:

- Dissolving the drug and excipients in a suitable solvent, generally water for injection (WFI).
- Sterilizing the bulk solution by passing it through a 0.22 micron bacteria-retentive filter.
- Filling into individual sterile containers and partially stoppering the containers under aseptic conditions.
- Transporting the partially stoppered containers to the lyophilizer and loading into the chamber under aseptic conditions.
- Freezing the solution by placing the partially stoppered containers on cooled shelves in a freeze-drying chamber or pre-freezing in another chamber.
- Applying a vacuum to the chamber and heating the shelves in order to evaporate the water from the frozen state.
- Complete stoppering of the vials usually by hydraulic or screw rod stoppering mechanisms installed in the lyophilizers.

Figure 6 presents a typical flowchart of the pharmaceutical application of lyophilization used for manufacturing parenteral products in vials.

There are many new parenteral products, including anti-infectives, biotechnology derived products, and in-vitro diagnostics which are manufactured as lyophilized products. Additionally, inspections have disclosed potency, sterility and stability problems associated with the manufacture and control of lyophilized products. It is recognized that there is complex technology associated with the manufacture and control of a lyophilized pharmaceutical dosage form. Some of the important aspects of these operations include: the formulation of solutions; the filling of vials and validation of the filling operation; sterilization and engineering aspects of the lyophilizer; scale-up and validation of the lyophilization cycle; and testing of the end product.

Product Type/Formulation

Products are manufactured in the lyophilized form due to their instability when in solution. Many antibiotics, such as some of the semi-synthetic penicillins, cephalosporins, and also some of the salts of

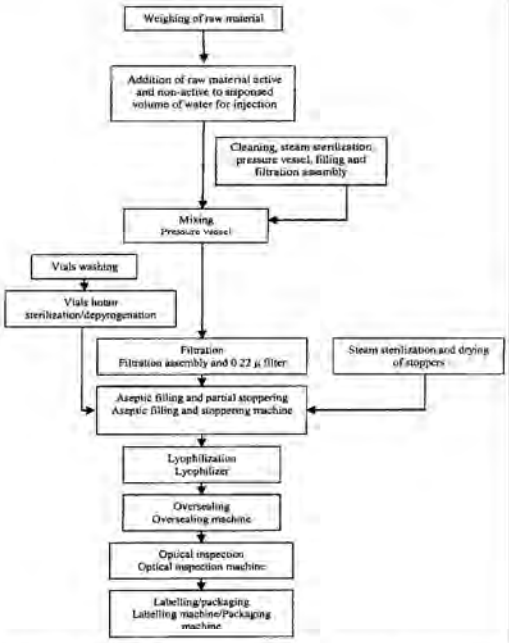


Figure 6. Typical flowchart of the pharmaceutical application of lyophilization – courtesy of the Hemoferm Group

erythromycin, doxycycline and chloramphenicol are made by the lyophilization process. Because they are antibiotics, a low bioburden of these formulations would be expected at the time of batching. However, some of the other dosage forms that are lyophilized, such as hydrocortisone sodium succinate, methylprednisolone sodium succinate and many of the biotechnology derived products, have no antibacterial effect when in solution.

For these types of products, the bioburden should be minimal and the bioburden should be determined prior to the sterilization of these bulk solutions prior to filling. Obviously, the batching or compounding of these bulk solutions should be controlled in order to prevent any potential increase in microbiological levels that may occur up to the time that the bulk solutions are filtered (sterilized). A concern with any microbiological level is the possible increase in endotoxins that may develop. Good practice for the compounding of lyophilized products would also include batching in a controlled environment and in sealed tanks, particularly if the solution is to be held for any length of time prior to sterilization.

Filling

The filling of vials that are to be lyophilized has some problems that are somewhat unique. The stopper

is placed on top of the vial and is ultimately seated in the freeze-dryer. As a result, the contents of the vial are subject to contamination until they are actually sealed.

Typically, vials to be lyophilized are partially stoppered by machine. However, some filling lines have been noted which utilize an operator to place each stopper on top of the vial by hand.

Once filled and partially stoppered, vials are transported to the lyophilizer by metal trays and loaded into the lyophilizer. The transfer and handling, such as loading of the lyophilizer, should take place under primary barriers, such as laminar flow hoods under which the vials were filled.

Lyophilization Cycle and Controls

After sterilization of the lyophilizer and aseptic loading, the initial step is freezing the solution. In some cycles, the shelves are at the temperature needed for freezing, while for other cycles, the product is loaded and then the shelves are taken to the freezing temperature necessary for product freeze. In those cycles in which the shelves are precooled prior to loading, there is concern for any ice formation on shelves prior to loading. Ice on shelves prior to loading can cause partial or complete stoppering of the vials prior to lyophilization of the product. A recent field complaint of a product in solution and not lyophilized was attributed to preliminary stoppering of a few vials prior to exposure to the lyophilization cycle. Unfortunately, the firm's 100% vial inspection failed to identify the defective vial.

Typically, the product is frozen at a temperature well below the eutectic point.

It is desirable after freezing and during primary drying to hold the drying temperature (in the product) at least 4–5°C below the eutectic point. Obviously, the manufacturer should know the eutectic point and have the necessary instrumentation to assure the uniformity of product temperatures. The lyophilizer should also have the necessary instrumentation to control and record the key process parameters. These include: shelf temperature, product temperature, condenser temperature, chamber pressure and condenser pressure. The manufacturing directions should provide for time, temperature and pressure limits necessary for a lyophilization cycle for a product. The monitoring of product temperature is particularly important for those cycles for which there are atypical operating procedures, such as power failures or equipment breakdown.

Electromechanical control of a lyophilization cycle has utilized cam-type recorder-controllers. However, newer units provide microcomputer control of the freeze drying process. A very basic requirement for a computer controlled process is a flow chart or logic. Typically, operator involvement in a computer controlled lyophilization cycle primarily occurs at the beginning. It consists of loading the chamber, inserting temperature probes in the product vials and entering cycle parameters such as shelf temperature for freezing, product freeze temperature, freezing soak time, primary drying shelf temperature and cabinet pressure, product temperature for the establishment of fill vacuum, secondary drying shelf temperature and secondary drying time.

For most cycles, stoppering occurs within the lyophilizer. Typically, the lyophilizer has some type of rod or rods (ram) which enter the immediate chamber at the time of stoppering. Once the rod enters the chamber, there is the potential for contamination of the chamber. However, since the vials are stoppered, there is no avenue for contamination of the vials in the chamber which are now stoppered. Generally, lyophilizers should be sterilized after each cycle because of the potential for contamination of the shelf support rods. Additionally, the physical act of removing vials and cleaning the chamber can increase levels of contamination.

In some of the larger units, the shelves are collapsed after sterilization to facilitate loading. Obviously, the portions of the ram entering the chamber to collapse the shelves enters from a non-sterile area. Attempts to minimize contamination have included wiping the ram with a sanitizing agent prior to loading. Control aspects have included testing the ram for microbiological contamination, testing it for residues of hydraulic fluid, and testing the fluid for its bacteriostatic effectiveness. One lyophilizer fabricator has proposed developing a flexible "skirt" to cover the ram.

FUTURE DEVELOPMENTS

Lyophilized products are currently being manufactured by the batch. While freeze- dried coffee is perhaps the only product that is manufactured on a continuous basis, it is really not a lyophilized product. Lyophilized coffee has a light yellow color rather than a dark brown, in addition, the methods used to manufacture coffee would hardly be acceptable for heat-sensitive injectable products (i.e., the shelf temperature is greater than 100°C). The remainder of this section will first consider a batch system and, in particular, the use of automatic loading and unloading systems. Based on a typical lyophilization process, the feasibility of a continuous freeze-drying system will be shown only to be possible if and only if there can be a major reduction in process time. A prototype continuous dryer will be described and its production output compared to that of a typical batch production dryer.

The Batch System

Automatic loading equipment capable of loading and unloading a 90 m dryer in 2–3 min [30] has become increasing popular in recent years. These systems are capable of loading either vials or vials in trays directly on the shelves of the dryer. One of the chief advantages of such loading devices is to significantly reduce the number of people in the clean room, and thereby approach the conditions of isolation technology during

the loading and unloading operation [31–33]. In this way, the probability of biological contamination of the product is greatly reduced.

The Continuous System

In 1975 there was considerable interest in continuous freeze systems. At that time, the system used trays to move the product from one stage to another. Since the trays did not come in direct contact with the shelves, the heat mechanism to the trays was mainly through radiation. The thermal radiation was produced from horizontal heating plates that were arranged to create isothermal zones through which the product trays would pass in a stack configuration. Each stack consisted of 15 trays [34].

It is of interest to note that, in his keynote address before the annual meeting of the Parenteral Drug Association on 3 November 1994, Polster [35] expressed concerns regarding the use of emerging technologies. He predicted that those manufacturers (of pharmaceutical products) who resisted technological advances would suffer at the hands of their competitors. He envisioned that in the coming 21st century, manufacturing would be performed using complete barrier technology. In the case of lyophilization, he predicted that the process would be continuous and require less than 8 h [35].

Table 3. Process time of a typical pharmaceutical or biotechnology lyophilization process

Process phase	Time
Filling and loading 37,000 vials at 120 vials/min	6 h
Freezing (ambient to –40°C)	12 h
Primary drying process	48 h
Secondary drying process	24 h
Backfilling and stoppering	1 h
Total process time	91 h

Consider what would be involved to lyophilize a pharmaceutical or biotechnology product in 10 ml vials on a continuous basis [36], using the following lyophilization process:

The hypothetical continuous system would consist of three separate chambers. The first chamber would be maintained at –40°C and at 1.01295 x 10^5 Pa. The second chamber would be maintained at the shelf temperature and be pressure defined for the primary drying process. Secondary drying would be conducted in a third chamber maintained at the final shelf temperature and pressure. The system would accommodate three trays of vials (each containing 168 vials) abreast.

With a system capable of filling 3 trays at a time, it would require approximately 6 hr to complete the filling process and another 6 hr to complete the freezing process. At a width capable of 3 trays across, the length of the freezing chamber would be 22 m. Assuming that each vacuum lock could accommodate only 3 trays abreast, then the length of the primary drying chamber would be 14 m, while the length of the secondary drying chamber would be 7. The estimated total length of such a system would be 44 m or about the height of a 7–story building.

Assuming that it would require 1 hr to load and unload a vacuum lock, the minimum time to transport all of the trays from the freezing chamber into the primary drying chamber would be 72 h. An additional 48 h would be necessary to complete primary drying and 24 hr to complete the secondary drying. The total process time would be 158 h, or 1.7 times the process performed in a batch freeze–dryer. The dimensions of the dryer could be reduced. For example, the length of the freezing chamber could be reduced by one–half; however, in so doing, the overall process time would be increased to 181 h.

If the above continuous dryer required sterilization, then the entire system would have to be fabricated as a pressure–coded vessel. This would certainly add to the cost of the dryer. The cost of such a freeze–dryer would certainly exceed the cost of present dryers having the same shelf capacity. When the additional processing time is factored in, the continuous dryer based on present lyophilization processes would hardly appear as an attractive alternative to present technology.

Only if the lyophilization process time could be reduced by a factor of 1/5th to 1/10th would a continuous freeze system be deemed feasible. Reducing the time of the lyophilization process would also lead to reduced dimensions for the dryer. Because of the increase in the drying rate, the cake would have to have a sufficient self– supporting structure during primary drying to withstand the force exerted by the flow of gas from the vial. Therefore, it is unlikely that a formulation having a solid content less than 2% would be a candidate for lyophilization in a continuous dryer.

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IZVOD

LIOFILIZACIJA – PROCES I INDUSTRIJSKA PRIMENA

(Pregledni rad)

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Ovaj rad daje osnovni pregled procesa liofilizacije i razmatranje principa procesa kroz osnove: formulacije, zamrzavanja, primarnog sušenja i sekundarnog sušenja. U ovom radu je liofilizacija definisana kao proces stabilizacije u kome se supstanca prvo zamrava i onda se sadržaj rastvarača redukuje sublimacijom (primarno sušenje), a zatim desorpcijom (sekundarno sušenje) do vrednosti koja više ne podržava hemijske reakcije i rast živih organizama. Načinjen je poseban osvrt na industrijsku primenu procesa a akcenat je stavljen na primenu liofilizacije u farmaceutskoj industriji i industriji hrane. Oprema koja se koristi za liofilizaciju kao i materijali koji se mogu liofilizirati su opisani dovoljno detaljno da pruže informacije o ograničenjima i prednostima liofilizacije. Predstavljeni su ekonomika procesa i poređenje sa konvencionalnim metodama sušenja. Istorijski pregled i trendovi u razvoju procesa, iz ugla industrije, daju uvid u to gde se liofilizacija nalazila i kuda je, danas, vodi njena široka industrijska primena.

Ključne reči: Liofilizacija • Liofilizator • Liofiliziranje • Primarno sušenje • Sekundarno sušenje • Komora • Polica • Kondenzator • Formulacija • Farmaceutska industrija • Industrija hrane •

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REMOVAL OF DISSOLVED OXYGEN FROM WATER: A COMPARISON OF FOUR COMMON TECHNIQUES

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Summary—Four common techniques for the removal of dissolved oxygen from water have been examined: boiling at 1 atm, boiling under reduced pressure, purging with N_2 and sonication under reduced pressure. After treatment, the residual oxygen in solution was analysed by the Winkler method. Nitrogen purging for 20–40 min at flow rate of 25 mL/s was found to be the most effective oxygen removal method. Boiling at 1 atm was found to be the least effective. None of the techniques evaluated here lead to complete removal of oxygen. The concentration of residual dissolved oxygen after purging for 20–40 minutes with nitrogen is 0.2–0.4 ppm.

The removal of dissolved oxygen from water is important when experimenting with redox sensitive reagents. For example, it is vital when conducting experiments with metal sulphide systems.[1] Dissolved oxygen can be removed from solution by both chemical and physical means. Chemical methods can be extremely effective. For example, addition of titanium (III) citrate removes dissolved oxygen and poises the E_H(pe) of a neutral solution anywhere between −480 mV (pe = −8.11) (equilibrium potential reported by Zehnder and Wuhrmann)[2] and −300 mV (pe = −5.07) (Rickard and Oldroyd, unpublished work). Addition of Ti(III) citrate has been used by Butler and Rickard[3] in the synthesis of framboidal pyrite, but it may not be suitable in experiments where titanium ions may catalyse reactions or sorb onto surfaces. In experiments where addition of a redox poise is undesirable, a physical method must be employed to remove oxygen. Although often used, the effectiveness of physical methods is rarely reported. The objective of this study is to evaluate the effectiveness of four physical methods that are in common usage. These are: purging with nitrogen, argon or a similar inert gas,[4] boiling at 1 atm,[5,6] sonication under "vacuum"[7] and boiling under "vacuum". Other methods that have been used in a few studies, but that are not evaluated here are degassing by passing water through a gas permeable plastic tube in a vacuum chamber[8] and boiling water while bubbling with nitrogen.[9]

EXPERIMENTAL

Methods for dissolved oxygen removal were as follows.

Nitrogen purging

One litre of deionized water was placed in a PTFE stoppered pyrex reaction kettle. A glass bubbler was fitted and the water was bubbled with prepurified grade nitrogen for 5, 20, 40 and 60 min at room temperature. No stirring was used because the mixing caused by the passage of the nitrogen was considered sufficient to keep the water well mixed. Nitrogen was allowed to escape by means of a small vent hole. No air trap was fitted because the high N_2 flow rates made such a device obsolete. The water purged for 20 min with pre-purified grade nitrogen was used to make all analytical reagents for this study; these reagents were preserved under N_2 during the balance of the investigation. The oxygen content of the prepurified nitrogen, according to the distributor, is < 5 ppm. In one set of experiments ultra high purity nitrogen with an oxygen content of < 0.5 ppm was used to purge water for 30 min. The flow rate of the nitrogen was measured by timed collection of the expelled gas in an upturned measuring cylinder full of water. The flow rate used in these experiments was approximately 25 ml of gas

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(at 1 atm) per sec. The deionized water used in this study has a resistance of 18 MΩ.

Boiling at 1 atm

One litre of deionized water was placed in a conical flask and boiled for 30 min on a hot plate in open air. The boiling water was then poured into a plastic bottle and the remaining air in the bottle was squeezed out before tightly capping. The bottle was then cooled in running tap water.

Boiling under reduced pressure

Five hundred millilitres of deionized water were placed in a stoppered Buchner flask and attached to a hand vacuum pump. A trap was placed between the Buchner flask and the pump to prevent significant quantities of water getting into the pump. After boiling the water for 30 min, the flask was sealed and cooled in running water.

Sonication under reduced pressure

The procedure was the same as for boiling under vacuum except that the Buchner flask was placed in a sonic bath and sonicated for periods of both 30 min and 1 hr.

Trace oxygen in water was analysed by a Winkler titration as described by Clesceri *et al.*[10] Addition of Mn(II) sulphate solution plus a strongly alkaline solution of NaI to the system results in the precipitation of manganese hydroxides of higher oxidation states. Iodide in solution is in turn oxidized by the manganese hydroxides to iodine. The amount of iodine produced is in proportion to the amount of oxygen in solution. Iodine was then titrated against a solution of sodium thiosulphate which had been previously standardized against $0.0250N \pm 0.0005N$ iodine solution. The overall reaction scheme for this method is summarized in Table 1.

All water samples for analysis were taken and treated in 250 ml conical flasks with ground glass stoppers. Analysis of the contents of each flask was carried out immediately after treatment.

Table 1. Summary of the reaction scheme for the Winkler method. After Hitchman[11]

$$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2$$
$$2Mn(OH)_2 + \tfrac{1}{2}O_2 - H_2O \rightarrow 2Mn(OH)_3$$
$$2Mn(OH)_3 + 6H^+ + 3I^- \rightarrow 2Mn^{2+} + I_3^- + 6H_2O$$
$$I_3^- \rightarrow I_2 + I^-$$
$$I_2 + S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

RESULTS

The results of this study are summarized in Table 2. The errors given with each oxygen concentration in Table 2 are the random errors due to uncertainties in the experimental technique. Reagents were prepared using water purged with nitrogen; when water prepared by that method was analysed it was assumed that any oxygen in the reagents represented neither an addition to, or subtraction from, the system. Thus it is true that any error due to oxygen in the Mn(II) sulphate and NaI solutions is appreciably smaller than the random error and so is negligible.

There is a systematic error present due to oxidation of Mn(II) to higher oxidation states in the solid during storage. This error was found to

Table 2. Oxygen concentrations of deionized water after treatment to remove dissolved oxygen[1]

Method	Time	Concentration O_2
N_2 purging	5 mins	0.47 ppm ± 0.05 ppm
(pre-purified grade,	5 mins	0.56 ppm ± 0.05 ppm
<5 ppm O_2)	5 mins	0.55 ppm ± 0.05 ppm
	20 mins	0.35 ppm ± 0.05 ppm
	20 mins	0.33 ppm ± 0.05 ppm
	20 mins	0.42 ppm ± 0.05 ppm
	20 mins	0.34 ppm ± 0.05 ppm
	20 mins	0.36 ppm ± 0.05 ppm
	40 mins	0.18 ppm ± 0.05 ppm
	40 mins	0.23 ppm ± 0.05 ppm
	60 mins	0.19 ppm ± 0.05 ppm
	60 mins	0.22 ppm ± 0.05 ppm
(Ultra high purity	30 mins	0.28 ppm ± 0.05 ppm
grade, <0.5 ppm O_2)	30 mins	0.24 ppm ± 0.05 ppm
	30 mins	0.30 ppm ± 0.05 ppm
	30 mins	0.28 ppm ± 0.05 ppm
Boiling at 1 atm	30 mins	1.20 ppm ± 0.05 ppm
	30 mins	1.21 ppm ± 0.05 ppm
	30 mins	0.87 ppm ± 0.05 ppm
	30 mins	0.93 ppm ± 0.05 ppm
	30 mins	0.77 ppm ± 0.05 ppm
	30 mins	0.75 ppm ± 0.05 ppm
	30 mins	1.00 ppm ± 0.05 ppm
	30 mins	0.85 ppm ± 0.05 ppm
Boiling under	30 mins	0.34 ppm ± 0.05 ppm
reduced pressure	30 mins	0.31 ppm ± 0.05 ppm
	30 mins	0.31 ppm ± 0.05 ppm
	30 mins	0.28 ppm ± 0.05 ppm
	30 mins	0.28 ppm ± 0.05 ppm
	30 mins	0.26 ppm ± 0.05 ppm
Sonication under	30 mins	6.92 ppm ± 0.05 ppm
reduced pressure	60 mins	0.67 ppm ± 0.05 ppm
	30 mins	2.38 ppm ± 0.05 ppm
	30 mins	1.18 ppm ± 0.05 ppm
	60 mins	0.55 ppm ± 0.05 ppm
	60 mins	0.49 ppm ± 0.05 ppm

[1]Concentrations derived from analysis of iodine produced directly from oxidation of manganese hydroxides by oxygen. Errors are the random errors due to uncertainty in experimental technique.

be +0.05 ppm O_2. Hence this represents a systematic over estimation of the O_2 content by +0.05 ppm. The data in Table 2 have been corrected to take this error into account.

DISCUSSION

Purging with nitrogen appears to be both a quick and effective procedure to scrub deionized water of dissolved oxygen. The optimum duration of the scrubbing at the flow rate used in these experiments is about 30–40 min (Fig. 1). Purging for longer periods, some workers bubble solutions for up to 1 day or more, will not further reduce the oxygen concentration. The kinetics of the degassing process depend upon the gas/liquid interface area, *i.e.* the removal of dissolved oxygen is faster when nitrogen is introduced as small bubbles. Therefore, a sintered glass bubbler to produce small N_2 bubbles is a critical element in a purging setup. The data also seem to indicate that the use of expensive ultra high purity grade nitrogen (<0.5 ppm O_2) does not measurably aid the removal of dissolved oxygen from water (Fig. 1). In this investigation no difference was found between the concentration of residual oxygen expected using pre-purified nitrogen (<5 ppm O_2) and that observed using ultra high purity nitrogen as the purge gas.

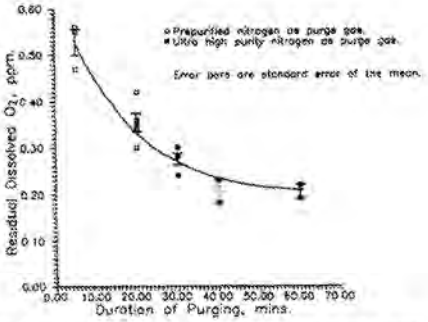


Fig. 1. Residual dissolved oxygen concentration after purging 1 l of distilled/deionized water with nitrogen at a flow rate of 25 ml/sec at 1 atm as a function of time. The initial oxygen concentration of the water was 8.5 ppm (measured with oxygen electrode) and the pH was near neutral. Most depletion occurred in the first 5 min. Further purging for 40 min to 1 hr resulted in a constant residual oxygen concentration of about 0.25 ppm. It is clear from this graph that extended durations of nitrogen purging do not noticeably further reduce the concentration of dissolved oxygen in solution. Purging with ultra high purity nitrogen is no more effective for the removal of dissolved oxygen than purging with prepurified grade nitrogen.

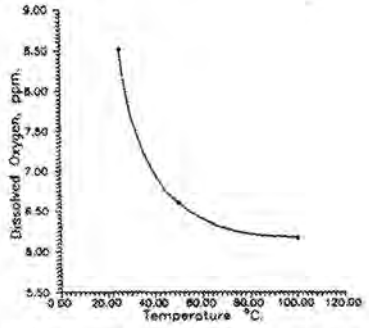


Fig. 2. Equilibrium dissolved O_2 concentration *vs* temperature. The data are calculated using Henry's law constants taken from the SOLTHERM database.[12] In all calculations it is assumed that P_{O_2} equals 0.21 atm. At 25°C, the equilibrium $[O_2]$ is 8.52 ppm, thus N_2 purging for 40 min represents a greater than 40 times reduction in $[O_2]$.

Boiling water at 1 atm is probably the most common of the methods under investigation here. The equilibrium concentration of oxygen in water at 100°C in contact with air is 6.17 ppm (calculated using the Henry's law constant for O_2 in water at 100°C[12] and assuming a P_{O_2} equal to 0.21 atm). This is significantly higher than the values found in this study. Therefore the removal of oxygen by this method clearly is not an equilibrium process. During the boiling process bubbles of water vapour, depleted in oxygen, are produced and it is with these that gas exchange takes place. Dissolved oxygen is entrained in the bubbles and then liberated to the atmosphere at the liquid surface. The liquid surface during vigorous boiling is extremely turbulent and so it is probable that a back reaction is taking place whereby atmospheric oxygen is able to redissolve in the water and is carried back into solution to be available once more for exchange at the liquid/bubble interface. This process may account for both the variability of the analytical results and the relative ineffectiveness of boiling at 1 atm as a method for the deoxygenation of water.

Boiling under reduced pressure is not only an effective method for removal of dissolved oxygen but it is also the most reproducible method studied here. Since oxygen is removed once liberated from the solution, the P_{O_2} of the gas phase in contact with the water is lower than for boiling at 1 atm. Hence the back reaction (uptake of O_2) will be of less importance and removal of dissolved oxygen more effective. More complete deoxygenation could

probably be achieved by the use of a more efficient vacuum pump or aspirator. Water deoxygenated by this method needs relatively little cooling before it can be used and use of a stronger pump would reduce this factor still further.

Sonication was used as an aid to bubble nucleation by Schoonen *et al.*[7] but the present study indicates that the process has little virtue other than to warm the water. Indeed the initial experiment resulted in an oxygen concentration of almost 7 ppm after 30 min; significant deoxygenation was only achieved after the water had reached 45°C. The boiling process produced in the sonic bath is less vigorous than that produced by a hotplate and this may account for the longer duration required for deoxygenation of the water. Clearly the sonication itself is of little value and similar results could probably be obtained using a water bath to maintain an evenly distributed temperature.

It should be noted that the results of this study are in sharp contrast to statements made by Hungate.[13] Hungate has reported that boiling for 1 min results in almost complete deoxygenation and that purging with gas for 30 min to 1 hr is less effective than boiling for this brief period. No raw data was presented by Hungate[13] to back up these conclusions and no final O_2 concentrations were reported. Clearly, from the present study, significantly better removal of dissolved oxygen can be achieved by purging or boiling under vacuum than by boiling at 1 atm.

In summary, the authors recommend use of pre-purified grade nitrogen, a sintered glass bubbler, high gas flow rates and an air-tight container (with a small vent hole to let the purge gas out) for effective removal of oxygen by this method (Fig. 3). This method may be further improved if O_2 is removed from the N_2 gas before passing it through the water. For example, oxygen may be removed by passing nitrogen over hot copper turnings. Nitrogen purging does also have the virtue that the solution is cool and ready for use immediately after oxygen removal, in contrast to other techniques reviewed here. A possible drawback of purging with nitrogen is that there will be nitrogen in solution after oxygen removal. At low temperatures (perhaps <150°C) nitrogen is essentially inert and should not be a factor unless reactions involving nitrogen fixing bacteria occur. However, at higher temperatures (>150°C) dissolved nitrogen may be involved in

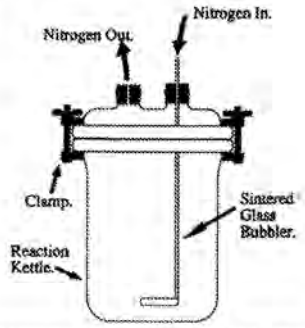


Fig. 3. Apparatus for N_2 purging of 1 l of deionized water. With this set-up it is possible to achieve $[O_2]$ equal to 0.2 ppm with N_2 purging for 40 min. The sintered glass bubbler is essential to obtain small bubbles, thus maximizing the surface area available for gas exchange. If a high N_2 flow rate is maintained then an O_2 trap is not needed at the gas outlet.

abiotic redox reactions. Thus for hydrothermal experiments, boiling under reduced pressure or perhaps nitrogen purging followed by boiling at reduced pressure should be employed.

It should be emphasized, however, that all the methods reviewed here leave some oxygen in solution and the complete elimination of oxygen from solution is not possible by any of the techniques evaluated in this study. In terms of the redox state of the water, the residual oxygen is significant. For example, 0.25 ppm residual O_2 at 25°C is equivalent to the equilibrium O_2 concentration in contact with an atmosphere with P_{O_2} equal to $10^{-2.2}$ atm. It is clear from Fig. 4 a pe/pH diagram, that even the best physical methods for the removal of dissolved oxygen cannot produce even a mildly reducing solution. Reduced conditions can only be obtained by using a redox poise, such as Ti(III) citrate. However, for some experiments it may be a challenge to find a redox poise that will only react with the dissolved oxygen and not with any other redox sensitive species or surface.

The inability to remove all dissolved oxygen with physical methods has important implications for the field of sulphide geochemistry. For example, the work by Sato[15] indicates that most sulphide minerals would be oxidized by the deoxygenated water produced in this study. Hence, exposure of most sulphide minerals to deoxygenated water is likely to lead to some change in their surface properties.[1] The residual dissolved oxygen becomes particularly important if the system under study has little or no

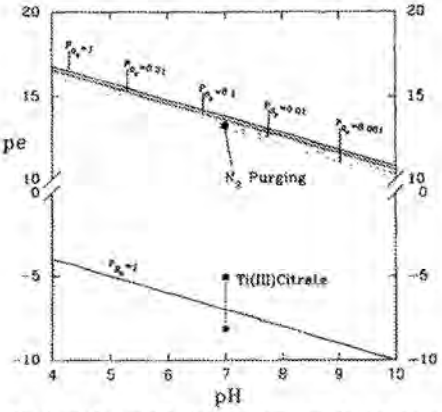


Fig. 4. pe (E_H) *vs* pH diagram illustrating the redox state attained via nitrogen purging to remove dissolved oxygen. For comparison, the pe range obtained by adding a Ti(III) citrate solution is also shown. The four physical methods evaluated in this study lead to oxygen depleted, but still oxidizing water. Data from Drever[14] were used to calculate this diagram. The diagram was calculated for 298 K and a total pressure of 1 atm. The pressure contours for O_2 and H_2 are given in atm. At 298 K, $E_H(mV) = 5.9*pe$.

redox buffer capacity. For example, in very dilute H_2S solutions, the remaining O_2 may react with a significant portion of the dissolved H_2S and radically change the solution pe (E_H) and the sulfur speciation.

Finally it should be noted that although purging with N_2 is an efficient method to remove dissolved oxygen from deionized water, it is a poor method to preserve solutions containing redox-sensitive species. Commercial grade nitrogen contains significant quantities of oxygen (<5 ppm for prepurified grades and <0.5 ppm for oxygen free and ultra high purity grades) and bubbling of N_2 through a solution containing redox sensitive species or minerals represents a constant advection of O_2 through the system. The O_2 in the gas is essentially scrubbed by the solution it passes through and will cause oxidation if bubbling is carried out over extended periods. For example, Pourbaix and Pourbaix[16] have shown, with reference to corrosion and aqueous sulphide systems, that occasional exposure to limited amounts of oxygen is not necessarily corrosive, but that continuous exposure of H_2S solutions to traces of oxygen can produce a significant pH drop possibly leading to corrosion. A better method to preserve such solutions would be to maintain them under a nitrogen or methane atmosphere, so allowing a minimum of exchange with oxygen impurities in the gas.

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Development and Evaluation of Lyophilized Product of Apo-Acetozolamide

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Abstract: Lyophilization or freeze drying involves the removal of water or other solvent from a frozen product by a process called sublimation followed by desorption.Lyophilization is a multistage operation in which, quite obviously, each step is critical. The main actors of this scenario are all well known and should be under strict to achieve a successful operation.Freeze-dried products for parenteral use are known as Powders for injection or infusion. As the stability of Acetazolamide in aqueous solution form was unstable it was formulated as Lyophilized product.The objective of the present study was to develop a stable lyophilized formulation of drug Apo-Acetazolamide for injection (500mg/vial) which is therapeutically equivalent to the reference listed product, DIAMOX.

OBJECTIVE AND PLAN OF THE WORK:

OBJECTIVE

- As the stability of Acetazolamide in aqueous solution form was unstable it was formulated as Lyophilized product.
- The objective of the present study was to develop a stable lyophilized formulation of drug Apo-Acetazolamide for injection (500mg/vial) which is therapeutically equivalent to the reference listed product, DIAMOX.

PLAN OF THE WORK

To achieve the ultimate goal of formulating lyophilized product of Apo-Acetazolamide, the present work was designed to address the following objectives: -

- Literature survey
- Procurement of API, Excipients and Packaging materials
- Innovator sample procurement and its characterization
- Preformulation studies of the drug
- Formulation of the injectable dosage form
- Development of Lyophilization cycle
- Lyophilization of the injectable dosage form
- Evaluation of lyophilized product
- Stability studies as per ICH guidelines

METHODOLOGY

- The experimental work starts with the literature survey of innovator product followed by the procurement and characterization of innovator product, sourcing of raw materials (API and excipients) and packaging materials.
- Based on the literature review, the proposed generic composition shall contain the following ingredients:

LIST OF RAW MATERIALS AND THEIR SOURCE:

S.No	INGREDIENT	VENDOR
1	Acetozolamide sodium equivalent to Acetozolamide	Aurobindo Pharma Ltd, India
2	Sodium hydroxide	Merck, Germany
3	Water for injection	Aurobindo Pharma Ltd, India

PROCUREMENT & CHARACTERIZATION OF INNOVATOR PRODUCT

Innovator drug product is procured from the respective market of source country and then they are characterized so as to check whether they conform to the product specifications.

Characterization of the innovator product includes:

a) Label Information

Strength, batch number, shelf life, storage instructions, reconstitution(if necessary) etc.

b) Primary Package Details

Vial type, dimensions, volume, fill volume, label size, head space analysis, etc.

c) Product Analysis

Appearance, assay, related substances, impurities concentration, sterility (endotoxin concentration.), pH etc

d) Stability study

PREFORMULATION STUDIES:

API Characterization:

- Preformulation involves the characterization of physical, chemical and microbiological attributes of the drug substance thus providing useful information so as to develop a safe, effective and stable dosage form.
- In the present study, the primary objective of the preformulation study is to ascertain that the drug substance complies with the specifications or pharmacopoeial standards.

The various tests conducted on the API during the preformulation study and the specifications set for the drug substance are tabulated below:

LIST OF PREFORMULATION TESTS AND THEIR SPECIFICATIONS:

TESTS	SPECIFICATIONS
DESCRIPTION	White to faintly yellowish-white, crystalline, odorless powder
SOLUBILITY	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly sluble in water.
IDENTIFICATION a) By IR	The IR spectrum of sample recorded, as KBr should exhibit transmission minima (absorption maxima)mat the same wavelenths to these in the spectrum obtained with the Acetazolamide working standard.

b) By chemical test	A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.
WATER CONTENT (%w/w, by KF, determined on 1g)	Not more than 3.0
pH (0.5g of sample in 25mL of carbon dioxide free water)	Between 9.1 and 9.6
HEAVY METALS (ppm)	Not more than 0.002
ASSAY (By HPLC, %w/w, as $C_4H_6N_4O_3S_2$ on anhydrous basis)	Not less than 98% and not more than 102%

RELATED SUBSTANCES (By HPLC, %w/w)	
5-amino-1,3,4-thiadiazole-2-thiol	Not more than 0.10
5-amino-1,3,4-thiadiazole-2-sulphnamide	Not more than 0.10
N-(5-sulphanyl-1,3,4-thiadiazol-2-yl)acetamide	Not more than 0.10
N-(5-chloro-1,3,4-thiadiazol-2-yl)acetamide	Not more than 0.10
N-(1,3,4-thiadiazol-2-yl) acetaide	Not more than 0.10
5-Acetamido-1,3,4-thiadiazole-2-sulphonic acid	Not more than 0.10
N-{5-[5-acetamido-1,3,4-thiadiazole-2yl]sulphonyl-1,3,4-thiadiazol-2-yl}acetamide	Not more than 0.10
Any unknown	Not more than 0.10
Total	Not more than 0.5
RESIDUAL SOLVENTS: (By GC-HS, µg/g)	
Methanol	Not more than 1000
Acetone	Not more than 1000
N,N-dimethyl formamide	Not more than 880
ADDITIONAL SOLVENT:	
Benzene	Not more than 2

BACTERIAL ENDOTOXINS (USP/mg of Acetazolamide)	Not more than 0.5
ABSORBANCE/ COLOUR OF SOLUTION (Dissolve 0.5g in 25mL of methanol, at 440nm, 1cm cell)	Not more than 0.2

LIST OF APPARATUS AND EQUIPMENTS:

S.No	NAME OF THE EQUIPMENT	MANUFACTURER	USE OF EQUIPMENT
1	Lyophilizer	Toffon Science and Technology Co Ltd. Shanghai China	To perform the process of Freeze drying
2	Freeze Drying Microscopy	Lyostat3, Biopharma technology limited.	To determine the collapse temperature(T_c)
3	Weighing balance	Sartorius, Japan	To weigh the raw materials and finished product
4	P^H meter	Thermoscientific (Model: Orion 3 star)	To find out the P^H of the product before and after Lyophilization
5	Filtration unit	Millipore	To clarify the drug solution
6	Modulated DSC	TA instruments (Model : Q 2000 series)	To determine the eutectic temperature of the drug
7	HPLC	Shimadzu HPLC LC 2010	To know the assay and related substances of the drug
8	KF titrator	Metrohm	To determine the water content of lyophilized drug
9	Particle size analyzer	Malvern Instruments. UK	To determine the size of particles

BACTERIAL ENDOTOXINS (USP/mg of Acetazolamide)	Not more than 0.5
ABSORBANCE/ COLOUR OF SOLUTION (Dissolve 0.5g in 25mL of methanol, at 440nm, 1cm cell)	Not more than 0.2

FORMULATION DEVELOPMENT:

As the Qualitative & Quantitative composition of Acetazolamide sodium for injection is given in the prescribing information leaflet of DIAMOX, the drug and excipients are selected based on the innovator formula.

PROTOTYPE FORMULA:

Sr. No.	Ingredients	Qty/Vial	Rationale	Present in innovator
1	Acetazalamide Sodium equivalent to Acetazolamide	500 mg	Active	Yes
3	Sodium Hydroxide	qs to pH	For pH Adjustment	Yes
4	Water for Injection	qs to 5mL	Solvent	Yes

MANUFACTURING PROCEDURE:

- Collect required batch size of Water for Injection in SS vessel; and cool the temperature to 4-8°C.
- Transfer 80% of Water for Injection from the above SS vessel to another SS vessel.
- Add weighed quantity of Acetazolamide with continuous stirring to get a clear solution.
- Check the pH and adjust the pH to 9.6 by using 1N NaOH.
- Make up the solution to required batch size with Water for Injection and check the final pH (Adjust if necessary).
- Filter the bulk solution through 0.2µ membrane filter.

- Fill the solution into to 10ml. USP Type I clear glass vials and half stopper it with double slotted chlorobutyl rubber stoppers and load for lyophilization as per optimized lyophilization cycle.
- After completion of the lyophilization, stopper the vials under vacuum and seal with aluminium flip-off seals.

EVALUATION OF LYOPHILIZED PRODUCT:

The lyophilized product was evaluated for the following formulation characteristics:

- Description
- Clarity of reconstituted solution
- Reconstitution time
- pH after reconstitution
- Water content
- Assay
- Related substances
- Particulate matter

STABILITY STUDIES:

STABILITY STUDIES AS PER ICH GUIDELINES:

Recommended Stability Condition/Station:

STATION	STORAGE CONDITION		
	40°C/75 % RH	30°C/65% RH	25°C/60% RH
1M	#↓	-	-
2M	#↓	-	-
3M	#*↓@	#↓	#↓
6M	#*↓@	#↓	#↓
9M	-	-	-
12M	-	#*↓	#*↓
24 M	-	-	@↓

\# - For analysis

* - BET (Bacterial Endotoxin Test)

↓ - Invert

@ - Reconstitution stability study

COMPATIBILITY STUDIES WITH PROCESS COMPONENTS:

Definition: Compatibility is a measurement of how stable a substance when it is in contact with another material. If there is no change in their physical and chemical properties of substance when it contacts with other materials is considered as compatible.

If changes happen in their physical and chemical properties of substances on contact with other material are considered as incompatible.

Types of Compatibility Studies:

- Filter compatibility
- Silicone Tubing compatibility
- SS Vessel compatibility
- 'O' ring compatibility

Study Design: This study can be in two ways.

Static Soak Method: In this study, the test filter shall be soaked in the drug product solution for longer period.

Dynamic Filtration: In this study, the liquid is continuously circulated through the filter membrane repeatedly.

Of these both methods, static soak method was used for the present study.

Experimental Procedure:

- The test shall be subjected to autoclaving and then use it for the compatibility studies.

- Place material in drug product solution and kept aside
- At regular time intervals (0, 4, 8, 12, 24 hours) withdraw the required amount of sample and perform analysis of it.

RESULTS AND DISCUSSION

EVALUATION OF INNOVATOR PRODUCT:

Product name : DIAMOX I.V
Drug substance : ACETAZOLAMIDE
Manufactured by : Bedford laboratories
Dosage form : Powder for injection
Storage condition : Store at room temperature
Batch number : AT2008
Expiry date : 15/1/2014

S.No	TEST PARAMETER	OBSERVATION
1.	**DESCRIPTION**	White freeze dried powder for injection
2.	**ASSAY**	101%
3.	**WATER CONTENT (%w/w)**	4.7
4.	**pH AFTER RECONSTITUTION**	9.6
5.	**RECONSTITUTION TIME**	54s
6.	**CONSTITUTED SOLUTION**	clear and free from visible particles
7.	**RELATED SUBSTANCES** **Total impurities (%w/w)**	0.5%
8.	**Bacterial endotoxins**	Not detected

PREFORMULATION STUDY:
API CHARACTERIZATION:

TESTS	SPECIFICATIONS	OBSERVATIONS
DESCRIPTION	White to faintly yellowish-white, crystalline, odorless powder	A white powder, crystalline, odorless powder
SOLUBILITY	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly soluble in water	Sparingly soluble in practically boiling water, slightly soluble in alcohol, very slightly soluble in water
IDENTIFICATION a) By IR	The IR spectrum of sample recorded, as KBr should exhibit transmission minima (absorption maxima)mat the same wavelenths to these in the spectrum obtained with the Acetazolamide workingstandard.	Concordant with that of Acetazolamide reference sample
B) By chemical test	A clear, bright yellow solution is produced. No heavy precipitate or dark brown color results after the mixing or heating.	Positive

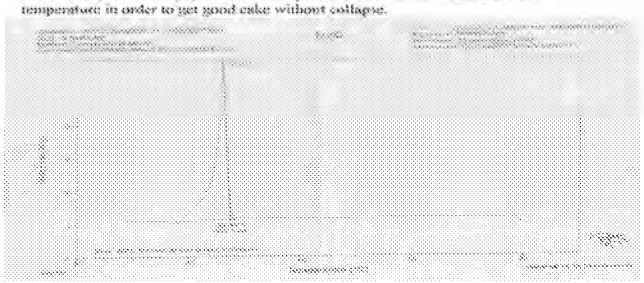
WATER CONTENT	Not more than 0.5	0.5
pH	Between 9.1 and 10	9.5
HEAVY METALS (ppm)	Not more than 0.002	Not more than 0.012
ASSAY	Not less than 99% and not more than 102%	99.5
RELATED SUBSTANCES Total	Not more than 0.5	Not detected
RESIDUAL SOLVENTS: Methanol Acetone N,N-dimethyl formamide **ADDITIONAL SOLVENT:** Benzene	Not more than 1000 Not more than 5000 Not more than 880 Not more than 2	Not detected 1002 440 Not detected
BACTERIAL ENDOTOXINS	Not more than 0.5	Not detected
ABSORBANCE OF SOLUTION	Not more than 0.2	0.124

From the above results, the API was found to be within its specifications given by the vendor and thus the drug substance Acetazolamide sodium can be used for the present study.

LYOPHILIZATION CYCLE DEVELOPMENT

DETERMINATION OF EUTECTIC TEMPERATURE

- The Eutectic temperature is determined by using *Modulated Differential Scanning Calorimeter* and was found to be -23.95°C.
- So in the process of lyophilization, the product should be freezed below this temperature in order to get good cake without collapse.



LYOPHILIZATION CYCLES

TRIAL 1

PRECOOLING

SL.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

SL.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (mbar)
1	-32	60	900	150
2	-10	60	480	150
3	0	60	120	150

CYCLE 2

PRECOOLING

SL NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180
3	-30	30	45
4	-45	30	120

PRIMARY DRYING

SL NO	FINAL TEMP.	RAMP DURATION(MIN)	SOAK DURATION (MIN)	PRESSURE (mbar)
1	-32	120	900	250
2	-10	30	300	100
3	0	120	300	150

SECONDARY DRYING

SL NO	FINAL TEMP.	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (mbar)
1	10	60	180	50
2	30	30	180	50
3	40	20	600	50

OBSERVATION: The moisture content observed was 10.1%. To reduce the water content a new modified cycle was taken in the next trial.

CYCLE 3

PRECOOLING

SNO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

S.NO	FINAL TEMP.	RAMP DURATION (MIN)	SOAK DURATION (MIN)	PRESSURE (mbar)
1	-25	120	300	250
2	-10	30	900	100
3	0	120	600	150

SECONDARY DRYING

S.NO	FINAL T°C	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (mbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 8.4%

CYCLE 4

PRE-COOLING

S.NO	FINAL T°C	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180
3	-30	30	45
4	-45	30	120

PRIMARY DRYING

S.NO	FINAL T°C	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (mbar)
1	-25	120	300	250
2	-10	30	300	100
3	0	120	300	150

SECONDARY DRYING

S.NO	FINAL T°C	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (mbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 6.36%.

CYCLE 5

PRE-COOLING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-30	120	300	250
2	-25	100	590	150
3	-15	10	600	100
4	0	120	600	150

SECONDARY DRYING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION	PRESSURE (µbar)
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 5.7%And time taken by the cycle is 67.80hrs≈2.8days.

To reduce the cycle duration a new modified cycle was taken in the next trial.

CYCLE 6

PRE-COOLING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)
1	-25.0	90	60
2	-45.0	90	180

PRIMARY DRYING

S.NO	FINAL T(°C)	RAMP DURATION(MIN)	SOAK DURATION(MIN)	PRESSURE (µbar)
1	-30	120	300	250
2	-25	120	590	150
3	-15	10	600	100
4	0	120	300	100

SECONDARY DRYING

S.NO	FINAL TEMP	RAMP DURATION(MIN)	SEAL DURATION	PRESSURE
1	10	60	240	50
2	30	30	240	50
3	40	20	240	50

OBSERVATION

- The moisture content was found to be 4.3% and the cycle time was reduced to 62.3 hrs from 67.80hrs.

EVALUATION OF LYOPHILIZED PRODUCT:

S.No	TEST PARAMETERS	SPECIFICATION	OBSERVATIONS
1.	**DESCRIPTION**	White freeze dried powder for injection	White freeze dried powder for injection
2.	**ASSAY**	90% - 110%	99.6%
3.	**WATER CONTENT (%w/w)**	≤ 7	≤ 4
4.	**pH AFTER RECONSTITUTION**	9.0	9.0
5.	**RECONSTITUTION TIME**	3 to	50sec
6.	**CONSTITUTED SOLUTION**	clear and free from visible particles	clear and free from visible particles
7.	**RELATED SUBSTANCES Total impurities (%w/w)**	≤ 5%	0.08%
8.	**Bacterial endotoxins (EU/mg)**	NMT 0.5 I.U/ml	Not detected

Based on the results obtained from the characterization product, it can be concluded that the drug product obtained is within its specified limits of the innovator product.

OBSERVATION:

- The manufacturing process adopted for the batch provided a clear colorless solution at the end of the bulk solution compounding. the lyophilized product complied with the tentative specifications for the product with respect to the tested critical parameters like assay, related substances ph and the water content was found to be 4.3%. And time taken by the cycle is 62.30hrs = 2.5days.
- The water content of this batch was reduced and the cycle time was reduced to 62.3 hrs from 67.80hrs.

CONCLUSION: The bulk solution manufacturing process used in this particular trial would be used as standard manufacturing process for all further studies unless started.

- As the water content of this batch was low, the same recipe has been proposed as final cycle for exhibit batch.

RECONSTITUTION STABILITY STUDIES:

RECONSTITUTION WITH 0.9% SODIUM CHLORIDE INJECTION:

S.No	Test parameter	Specification	Reconstitution with 0.9%NaCl			
			0 hrs	6 hrs	12 hrs	24 hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear colour less	Clear colourless	Clear colour less
2.	**Assay**	98-102%	99.7	100.4	100.1	98.9
3.	**pH**	9.6	9.62	9.61	9.62	9.63
4.	**Related substances Total impurities**	NMT 0.5%	0.03	0.2	0.23	0.3
5.	**Particulate matter (per container)**	≥25µ - 600/unit	6	14	213	42
		≥10µ - 6000/unit	131	180	190	430

From the above results, it can be concluded that **0.9% SODIUM CHLORIDE INJECTION** for reconstitution.

COMPATIBILITY STUDIES WITH PROCESS CONTAINERS:

FILTER COMPATIBILITY STUDY:

S.No	Test parameter	Specification	Initial	PVDF Filter compatibility			
				4 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colou r less
2.	**Assay**	90-102%	99.3	99.2	99.4	99.1	98.9
3.	**pH**	9-10	9.62	9.63	9.63	9.62	9.63
4.	**Related substances Total impurities**	NMT 1.0%	0.03	0.04	0.08	0.08	0.08
6.	**Particulate matter (per container)**	≥10µ - 6000	224	310	320	325	957
		≥25µ - 600	6	24	26	32	62

From the above results, it can be concluded that PVDF filter can be used for the purpose of the filtration during the experimental work.

MULTIPOINT COMPATIBILITY STUDY:

S.No	Test parameter	Specification	Initial	Pharma Pure compatibility (storage at 2-8 °C)			
				8 hrs	48hrs	120hrs	240hrs
1	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Small particles observed	Small particles observed
2.	**Assay**	98-102%	101	99.7	98.2	98.3	97.9
3.	**pH**	9.6	9.62	9.63	9.64	9.63	9.64
5.	**Related substances Total impurities**	NMT 0.5%	0.03	0.01	0.1	0.1	0.1
6.	**Particulate matter (per container)**	≥10µ - 6000	224	367	585	1064	2020
		≥25µ - 600	6	108	120	128	310

2. PHARMA 50 TUBE:

S.No	Test parameter	Specification	Initial	Pharma 50 compatibility (storage at 2-8 °C)			
				0 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Small particles observed	Small particles observed
2.	**Assay**	98-102%	101	99.3	99.2	99.2	98.9
3.	**pH**	9-10	9.62	9.62	9.63	9.63	9.63
4.	**Related substances Total impurities**	NMT 1.0%	0.03	0.07	0.08	0.08	0.09
5.	**Particulate matter (per container)**	≥10µ - 6000	224	260	280	630	920
		≥25µ - 600	6	17	22	33	43

From the above results, it can be inferred that the silicon tubings should not be in contact with the drug solution for more than 8hrs because beyond this period particle growth was being observed in the drug solution.
Of both the tubings, Pharma 50 has shown better results than Pharma Pure relating to particulate matter. So Pharma 50 can be used during the experimental work.

3. SS VESSEL COMPATIBILITY STUDY:

S.No	Test parameter	Specification	Initial	SS Vessel compatibility (storage at 2-8 °C)			
				0 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	**Assay**	98-102%	101	99.6	99.5	99.1	98.9
3.	**pH**	9-10	9.62	9.64	9.64	9.64	9.65
4.	**Related substances Total impurities**	NMT 1.0%	0.03	0.04	0.04	0.07	0.08
5.	**Particulate matter (per container)**	≥10µ - 6000	224	180	220	280	1020
		≥25µ - 600	6	4	17	32	81

The above results indicate that SS Vessel was found to be compatible with the drug solution throughout the study period and therefore it can be used for the holding the drug solution before loading on to the lyophilizer.

'O' RING COMPATIBILITY STUDY:

1. SILICON 'O' RING:

S.No	Test parameter	Specification	Initial	Silicon O ring compatibility (storage at 2-8 °C)			
				0 hrs	8hrs	12hrs	24hrs
1.	**Description**	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	**Assay**	98-102%	99.8	99.8	99.4	99.3	99.2
3.	**pH**	9-10	9.62	9.63	9.63	9.64	9.63
4.	**Related substances Total impurities**	NMT 1.0%	0.03	0.04	0.03	0.06	0.1
5.	**Particulate matter (per container)**	≥10µ - 6000	224	223	240	441	820
		≥25µ - 600	6	7	11	22	72

2. PTFE 'O' RING CONDITIONS ETC:

S.No	Test parameter	Specifications	Initial	PTFE O ring integrability Storage at 2-8 °C)			
				4 hrs	8hrs	12hrs	24hrs
1.	Description	Clear, colour less solution	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less	Clear, colour less
2.	Assay	98-102%	101	99.8	99.5	99.3	98.7
3.	pH	9.6	9.63	9.62	9.63	9.63	9.62
4.	Related substances Total impurities	NMT 0.5%	0.03	0.07	0.08	0.08	0.03
5.	Particulate matter (per container)	≥10µ - 6000	232	289	312	351	423
		≥25µ - 600	5	17	7	19	15

Even though both the 'O' rings had shown satisfactory results with respect to the assay, pH and related substances, Silicon 'O' ring had liberated more particulate growth than PTFE 'O' ring during the analysis period. So, PTFE 'O' ring can be selected for the present study.

Table : Stability data of 1st and 2nd months under accelerated storage condition

S.No	Test parameter	Specifications	Initial	40°C/75 % RH	
				1st Month	2nd Month
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.7	99.4	98.2
3.	pH after reconstitution	9.6	9.63	9.63	9.63
4.	Reconstitution time	NMT 60s	30s	32s	35s
5.	Constituted solution	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	Related substances Total impurities	NMT 0.5%	0.03	0.14	0.15
7.	Particulate matter (per container)	≥10µ - 6000	231	254	298
		≥25µ - 600	8	19	34
		≥50µ	0	2	5

Table : Stability data of 3rd and 6th months under accelerated storage condition

S.No	Test parameter	Specifications	40°C/75 % RH			
			3rd Month		6th Month	
			At the time of reconstitution	12hrs after reconstitution	At the time of reconstitution	12hrs after reconstitution
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	93.7	89.2	89.1	98.9
3.	pH after reconstitution	9.6	9.7	9.6	9.9	9.6
4.	Reconstitution time	NMT 60s	30s	50s	55s	55s
5.	Constituted solution	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	Related substance, total impurities	NMT 0.5%	0.01	0.02	0.21	0.3
7.	BET	NMT 0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg	<0.5 EU/mg
8.	Particulate matter (per container)	≥10µ -6000	315	369	422	531
		≥25µ - 600	56	69	89	98
		≥50µ	11	18	35	52

The accelerated stability study results confirm that all the test parameters were within the specifications.

LONG TERM AND INTERMEDIATE STORAGE CONDITION STABILITY STUDY

S.No	Test parameter	Specifications	25°C/60% RH		30°C/65% RH	
			3rd Month	6th Month	3rd Month	6th Month
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.6	99.1	99.5	98.9
3.	pH after reconstitution	9.6	9.62	9 63	9.63	9.64
4.	Reconstitution time	NMT 60s	51s	52s	50s	53s
5.	Constituted solution	Clear, colour less solution free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution free from visible particles	Clear, colour less solution, free from visible particles
6.	Related substances, Total impurities	NMT 0.5%	0.01	0.02	0.2	0.3
7.	Particulate matter test by Light Obscuration test method (per container)	≥10μ-6000	355	365	411	525
		≥25μ - 600	59	86	90	81
		≥50μ	15	31	20	34

The above results confirm that the developed lyophilized drug was stable at long term storage condition (25°C/60% RH) and intermediate storage condition (30°C/65% RH) for a period of 6 months.

THERMAL CYCLING STUDY

The product was evaluated for the effects of temperature variation on the product when subjected to temperature condition.

S.No	Test Parameter	Specifications	Initial	Cycle 3
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.6	98.9
3.	pH after reconstitution	9.6	9.7	9.6
4.	Reconstitution time	NMT 60s	50	51s
5.	Constituted solution	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
6.	Related substances Total impurities	NMT 0.5%	0.01	0.2
7.	BET	NMT 0.075 EU/mg	<0.075 EU/mg	<0.075 EU/mg
8.	Particulate matter (per container)	≥10μ - 6000	158	221
		≥25μ - 600	2	5
		≥50μ	0	1

TEMPERATURE EXCURSION STUDY

S.No	Test parameter	Specifications	Initial	2 days at -20°C	2 days at 60°C
1.	Description	White freeze dried powder	White freeze dried powder	White freeze dried powder	White freeze dried powder
2.	Assay	98-102%	99.7	99.4	98.2
3.	Water content by KF	NMT 7.0%	1.59	4.27	2.10
4.	pH after reconstitution	9.2-10	9.7	99.1	99.9
5.	Reconstitution time	NMT 60s	50s	52s	50s
6.	Constituted solution	Clear, colour less solution free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles	Clear, colour less solution, free from visible particles
7.	Related substances Total impurities	NMT 0.5%	0.01	0.04	0.2
9.	Particulate matter (per container)	≥10μ - 6000	181	115	320
		≥25μ - 600	6	5	8
		≥50μ	0	0	2

OBSERVATION:

From the results of the Thermal Cycling and Temperature Excursion study we can confirm that the developed lyophilized drug product will be stable when it was subjected to or cycled through temperature conditions that

simulate the short term excursions outside the proposed label storage conditions which are likely to be encountered by the drug product during distribution.

So, it can be concluded that the drug product can be shipped between different climatic conditions without any change in its quality.

SUMMARY AND CONCLUSION

Before the development of the formulation, preformulation studies were conducted. In that solubility studies of the drug product, was determined the suitable conditions for the development of an optimized product.

Eutectic temperature was determined by using DSC and collapse temperature was determined which is the key parameter in the development of lyophilisation cycle recipe. Depending upon the value of eutectic temperature the recipes of the lyophilisation cycles were developed and optimized cycle was decided by trial and error method. Among all the above formulations, cycle 6 produces excellent product which is having desired qualities of the lyophilized product. This product was then compared with the innovator. And product was then subjected to various tests like reconstitution stability, stability studies as per ICH guidelines.

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Freeze Drying

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INTRODUCTION

Freeze drying, also termed "lyophilization," is a drying process employed to convert solutions of labile materials into solids of sufficient stability for distribution and storage. A typical production scale freeze dryer consists of a drying "chamber" containing temperature-controlled shelves, which is connected to a "condenser" chamber via a large valve. The condenser chamber houses a series of plates or coils capable of being maintained at very low temperature (i.e., less than −50°C). One or more vacuum pumps in series are connected to the condenser chamber to achieve pressures in the range of 0.03–0.3 Torr in the entire system during operation. A commercial freeze dryer may have 10–20 shelves with a total load of the order of 50,000 vials. The objective in a freeze-drying process is to convert most of the water into ice in the *freezing stage*, remove the ice by direct sublimation in the *primary drying stage*, and finally remove most of the unfrozen water in the *secondary drying stage* by desorption. The water removed from the product is reconverted into ice by the condenser.

In a typical freeze-drying process, an aqueous solution containing the drug and various formulation aids, or "excipients," is filled into glass vials, and the vials are loaded onto the temperature-controlled shelves. The shelf temperature is reduced, typically in several stages, to a temperature in the vicinity of −40°C, thereby converting nearly all the water into ice. Some excipients, such as buffer salts and mannitol, may partially crystallize during freezing, but most "drugs," particularly proteins, remain amorphous. The drug and excipients are typically converted into an amorphous glass also containing large amounts of unfrozen water (15–30%) dissolved in the solid (i.e., glassy) amorphous phase. Thus, most of the desiccation actually occurs during the freezing stage of the freeze-drying process. After all water and solutes have been converted into solids, the entire system is evacuated by the vacuum pumps to the desired control pressure, the shelf temperature is increased to supply energy for sublimation, and primary drying begins. Due to the large heat flow required during primary drying, the product temperature runs much lower than the shelf temperature. The removal of ice crystals by sublimation creates an open network of "pores," which allows pathways for escape of water vapor from the product. The ice–vapor boundary (i.e., the boundary between frozen and "dried" regions) generally moves from the top of the product toward the bottom of the vial as primary drying proceeds. Primary drying is normally the longest part of the freeze-drying process. Primary drying times of the order of days are not uncommon, and in rare cases, weeks may be required for a combination of poor formulation and suboptimal process design. Although some secondary drying does occur during primary drying (i.e., desorption of water from the amorphous phase occurs to a limited extent once the ice is removed from that region), the start of secondary drying is normally defined, in an operational sense, as the end of primary drying (i.e., when all ice is removed). Of course, because not all vials behave identically, some vials enter secondary drying whereas other vials are in the last stages of primary drying. When the judgment is made that all vials are devoid of ice, the shelf temperature is typically increased to provide the higher product temperature required for efficient removal of the unfrozen water. The final stages of secondary drying are normally carried out at shelf temperatures in the range of 25–50°C for several hours. Here, because the demand for heat is low, the shelf temperature and the product temperature are nearly identical.

As freeze-drying plants are very expensive and process times are often long, a freeze-dried dosage form is relatively expensive to produce. Indeed, because of both cost and ease of use, a ready-to-use solution is the preferred option for a parenteral dosage form, particularly if the solution can withstand terminal heat sterilization. When an aqueous solution does not have sufficient stability, the product must be produced in solid form. At least for small molecules, stability normally increases in the following order: solution < glassy solid < crystalline solid;[1–3] this is likely a result of restricted motion in solids with the high degree of order in the crystalline solid limiting reactivity even further. As many pharmaceuticals cannot be produced on a commercial scale by crystallization, a glassy solid may be the only solid-state option.

Freeze drying[4–7] and spray drying[8–10] are drying methodologies in common use in the pharmaceutical industry, and are suitable for the production of

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glassy solids. Freeze drying is a low-temperature process. In general, a formulation can be dried to 1% water or less, without any of the product exceeding 30°C. Thus, conventional wisdom states that freeze-drying is less likely to cause thermal degradation than a high-temperature process such as spray drying. Historically, freeze-drying is the method of choice for products intended for parenteral administration. Sterility and relative freedom from particulates are critical quality attributes for parenterals. Largely because the solution is sterile filtered immediately before filling into the final container, and further processing is relatively free of exposure to humans, a freeze-drying process maintains sterility and particle-free characteristics of the product much more easily than do processes that must deal with dry powder handling issues, such as dry powder filling of a spray-dried or bulk-crystallized powder. Indeed, with modern robotics automatic loading systems,[11] humans can be removed from the sterile processing area entirely, at least in principle. Furthermore, as the vials are sealed in the freeze dryer, moisture control and control of headspace gas can easily be controlled, an important advantage for products whose storage stability is adversely affected by residual moisture and/or oxygen. As the critical heat and mass transfer characteristics for freeze-drying are nearly the same at the laboratory scale as in full production, resolution of scale-up problems tends to be easier for a freeze-drying process than for spray drying, at least in our experience. Also, development of a freeze-dried product requires less material for formulation and process development, a particularly important factor early in a project.

While freeze-drying has a long history in the pharmaceutical industry as a technique for stabilization of labile drugs, including proteins, many proteins suffer irreversible change, or degradation, during the freeze-drying process.[12–16] Even when the labile drug survives the freeze-drying process without degradation, the resulting product is rarely found perfectly stable during long-term storage, particularly when analytical techniques with a sensitivity to detect low levels of degradation (≈0.1%) are employed. Both small molecules[1–3,17] and proteins[18–21] show degradation during storage of the freeze-dried glass. In some cases, instability is serious enough to require refrigerated storage.[18,19,22]

Stability problems are most often addressed by a combination of formulation optimization and attention to process control. Lyoprotectants are added for stability during the freeze-drying process as well as to provide storage stability, and the level and type of buffer is optimized. Optimization of the freezing process may be critical; control of product temperature during drying is critical for products that tend to suffer cake collapse during primary drying, and control of residual moisture is nearly always critical for storage stability. Formulation and process are interrelated: A bad formulation can be nearly impossible to freeze dry, and even with a well designed formulation, a poorly designed process may require more than a week to produce material of suboptimal quality. Although blind empiricism may, in time, yield an acceptable formulation and process, an appreciation for the materials science of amorphous systems and some understanding of heat and mass transfer relevant to freeze-drying are needed for efficient development of freeze-dried products. Obviously, one also requires at least a phenomenological understanding of the major degradation pathways specific to the drug under consideration.

The objective of this article is to present the scientific and engineering fundamentals most useful in the development of formulations and processes for the manufacture of freeze-dried pharmaceuticals. Generalizations are illustrated with specific examples from the literature, but no attempt is made to survey all published works. Most of the section on the freeze-drying process applies equally well to small molecules and proteins, whereas most of the section on formulation and stability is specific to proteins.

THE FREEZE-DRYING PROCESS

Freezing

Freeze concentration

The objective of freezing is to remove most of the water from the system by formation of ice and to convert all solutes into solids, either crystalline solids or a glass. Once the sample is a solid, primary drying may begin. As a sample is cooled, the system remains liquid well below the equilibrium freezing point, but with sufficient supercooling, nucleation of ice proceeds rapidly and growth of ice crystals begins. As liquid water converts to ice, all solutes are concentrated in the regions between ice crystals, ultimately concentrating until they crystallize or until the system increases in viscosity sufficiently to transform into a solid amorphous system, or glass. The example shown in Fig. 1 is representative of a solute that does not crystallize during freeze-drying. The system supercools about 10°C to a temperature of −15°C before ice nucleation becomes rapid enough to generate crystals of ice. The evolution of heat caused by the sudden crystallization of ice increases the sample temperature to roughly the equilibrium freezing point (−5°C). The sudden ice crystallization also results in a small but sharp decrease in the percentage of water in the solute phase (given on the right vertical axis). After the initial ice nucleation and crystallization at 10 min, the product cools with continuous conversion of water to ice, thereby

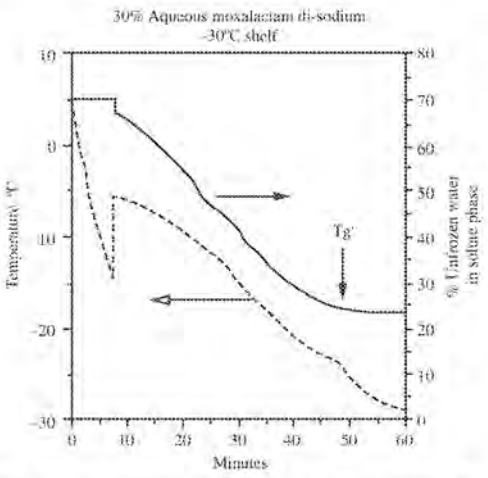


Fig. 1 Freeze concentration in an amorphous system. The product temperature is shown as a broken line whereas the percentage of unfrozen water is shown as a solid line. (From Ref.[6].)

decreasing the amount of water in the solute phase and increasing the concentration of solute in the remaining solution. When the product temperature reaches about −24°C, denoted as T_g', the concentration of water in the solute phase has been reduced to about 24%, and further reduction in water concentration is curtailed on the time scale of freezing, even though the sample temperature decreases further to about −30°C at the end of the freezing process at 60 min. Here, the solute phase has been concentrated from about 30% solute to about 76% solute, and most of the water has been separated from the solute phase as ice. Had the initial concentration been much lower, say 1%, the final concentration would still be 76%, although the freezing profile would differ quantitatively from that shown in Fig. 1. Clearly, most of the desiccation in a freeze-drying process occurs during the freezing process. While desiccation is indeed the objective, it must be recognized that concentration of all solutes during freezing will dramatically increase the probability of bimolecular collisions, which could produce unexpected instability in the partly frozen system. If a system, initially at 1% solute, is susceptible to a second-order degradation reaction with an activation energy of 25 kcal/mol, a reduction in temperature from 5°C to −24°C would reduce the rate constant by a factor of 200, but an increase in concentration from 1% to 76% would increase the concentration factor in the rate equation by a factor of 5800, yielding a factor of 29 net increase in reaction rate. Although this example is an oversimplification, it is significant to note that increases in second-order degradation rates have been observed in model systems during freeze concentration.[23]

T_g' is the temperature at which a sharp increase in baseline occurs during a differential scanning calorimeter (DSC) scan of a frozen solution, suggesting a sharp increase in heat capacity, and has been referred to as the glass transition temperature of the maximally concentrated freeze concentrate.[24] A sharp decrease in electrical resistance of the frozen system is noted at the same temperature.[25] Structural collapse of a cake structure in the dried region, indicating viscous flow, occurs during primary drying at the collapse temperature, denoted by T_c, which is only slightly higher than T_g'.[25] It is clear that T_g' corresponds to the temperature at which mobility in the system is manifested on an experimental timescale. Effectively, the system behaves as a solid below T_g'.

Various electrolytes, such as buffer salts and NaCl, if present in the formulation, will also concentrate during the freezing process. Such exposure to high concentrations of electrolytes (i.e., about 6 molal NaCl during freezing a 0.15 M NaCl solution) might contribute to the destabilization of the native conformation, thereby leading to degradation during freezing.

Crystallization of excipients and consequences

In the case of protein drugs, the drug does not crystallize, but other solute components may (or may not) crystallize, depending on their nature and concentration, other formulation components, and the details of the freezing process. High initial concentrations and slow freezing tend to favor crystallization. Depending on the intended role of the excipient, crystallization may be desirable or undesirable, and it may be important just when in the process crystallization does occur. For example, if mannitol is intended only as a bulking agent, and is not expected to play a role in stabilization of the drug, crystallization is desirable as crystalline mannitol can easily be freeze dried at high temperatures to give an elegant product, meaning a short freeze-drying cycle and freedom from product defects such as collapse. Here, the drug form is that of an amorphous coating on the crystalline mannitol, with stability properties normally close to that of a system freeze dried without the mannitol. If mannitol remains amorphous during freezing and crystallizes as the product temperature is increased in early primary drying, extensive vial breakage can occur.[26] Conversely, if mannitol is intended to serve as a stabilizer during drying and/or storage, crystallization is undesirable. Complete crystallization yields a system equivalent to a physical mixture of glassy drug particles and crystalline mannitol, with no opportunity for a stabilizing interaction except at the phase boundary between the

particles, no dilution of drug molecules, and no separation of "reactants" in the protein phase from the protein molecules. In short, any plausible stabilization mechanism requires that at least some of the mannitol remain molecularly dispersed in the amorphous drug phase. Of course, just as crystalline mannitol has some solubility in any liquid, mannitol will have a nonzero thermodynamic solubility in the drug phase. Thus, even if mannitol crystallization proceeds to thermodynamic equilibrium, a small amount of mannitol will remain in the drug phase. However, most of the stabilization potential of the mannitol will be lost upon crystallization. As mannitol does tend to crystallize nearly completely when present as the major formulation component, mannitol is generally a poor choice as a stabilizer for freeze drying.

Although buffers are included in a formulation to maintain constant pH, selective crystallization of the less soluble buffer component during freezing can result in massive pH shifts in the freeze concentrate. Fig. 2 shows the pH changes observed during equilibrium freezing of several pure buffer systems.[27] Freezing was carried out while seeding with ice and crystalline buffer salts, so the data represent near-equilibrium behavior. Over the temperature range studied, the citrate buffer system showed no significant pH shift. The sodium phosphate buffer system shows a dramatic decrease in pH of about 4 pH units due to crystallization of the basic buffer component, $Na_2HPO_4 \cdot 2H_2O$. Conversely, the potassium phosphate system shows only a modest increase in pH of about 0.8 pH unit. Under nonequilibrium conditions (i.e., no seeding) and with lower buffer concentrations, the degree of crystallization is less, and the resulting pH shifts are moderated.[28] Table 1 shows data accumulated[29,30] during freezing of phosphate buffer solutions in large volumes at cooling rates intended to mimic freezing in vials. For the concentrated buffer solutions (100 mM), the frozen pH values are close to the equilibrium values given in Fig. 2. However, lowering the buffer concentration by an order of magnitude considerably reduces the pH shift observed during freezing. It should also be noted that the small pH shift for potassium phosphate buffer noted in Fig. 2 is a result of the starting pH being 7.5. As shown in Table 1, if the initial pH is 5.5, the 100 mM potassium phosphate buffer increases by 3.1 pH units during freezing. In short, the frozen system pH is near 8.7, regardless of whether the initial pH is 7.0 or 5.5. Clearly, if drug stability is sensitive to pH shifts, buffer crystallization must be avoided. In our experience, the best solution is to formulate such that the weight ratio of buffer to other solutes is very low.[18,22] However, the precise meaning of "very low" varies with the nature and amount of the other solutes as well as with the nature of the buffer.

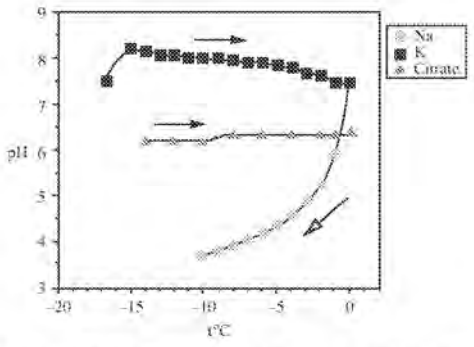


Fig. 2 Shifts in pH during freezing arising from buffer salt crystallization. Near-equilibrium conditions were achieved by seeding with ice and salt. circles: sodium phosphate; squares: potassium phosphate; triangles: citrate. (Data from Ref.[27].)

Table 1 Shifts in pH during non-equilibrium freezing with phosphate buffer systems

Concentration (mM)	Initial pH	Frozen pH	Δ pH
Sodium phosphate buffer			
100	7.5	4.1	−3.4
8	7.5	5.1	−2.4
Potassium phosphate buffer			
100	7.0	8.7	+1.7
100	5.5	8.6	+3.1
10	5.5	6.6	+1.1

(From Refs.[29,30].)

Instability during freezing and T_g'

Most small molecules are stable during the freezing process, even though storage stability may be marginal. Proteins show a wide range of formulation-sensitive instability. Some proteins survive freezing with little or no measurable loss in activity, whereas others are irreversibly deactivated by the freezing process.[12–21,31–33] An environment quite different from a dilute aqueous solution is created during freezing. All solute species are dramatically concentrated, ionic strength increases, the pH may shift, and above all, hydrophobic interactions that stabilize the native conformation in water are reduced or eliminated as bulk water is removed from the protein phase. In addition, just as proteins undergo thermal denaturation at elevated temperature, proteins also undergo spontaneous unfolding at very low temperature, called "*cold denaturation*".[34,35] Estimated cold denaturation temperatures are often below the T_g' of a protein formulation and therefore of questionable relevance

to freeze-drying. However, these estimates are based upon thermodynamic parameters measured in dilute aqueous solutions. The impact of perturbations caused by freeze concentration is largely unknown.

Because of these many freezing stresses that may decrease the free energy of denaturation, thermodynamic destabilization of the native conformation during freezing is not surprising. Indeed, it appears surprising that all proteins do not spontaneously unfold and degrade during freezing. However, for a destabilized protein to unfold and engage in subsequent irreversible reactions, the rate of unfolding must be fast relative to the timescale of freezing. Even in dilute aqueous solution at room temperature, protein unfolding may involve time constants of the order of hours.[36,37] In the freeze concentrate approaching T_g', at temperatures $\approx$30°C lower than room temperature and viscosities about 10 orders of magnitude greater than in a dilute aqueous solution, one would expect greatly reduced unfolding rates, perhaps sufficiently reduced to prevent degradation during freezing. Kinetic studies of unfolding near T_g' would resolve this question. Unfortunately, such data are not available. However, it has been argued on theoretical grounds that rate processes, in general, should be slowed greatly as the system temperature approaches a glass transition temperature.[33] Experimental studies of three different reactions in frozen maltodextrin systems lend support to this view, although not all reactions show the same strong temperature dependence near a glass transition.[38] As a general rule, it would seem prudent to minimize the time a protein spends in a freeze concentrate above T_g' (i.e., minimize freezing time), and primary drying should be carried out near or below T_g'. However, these precautions do not always guarantee stability.

Optimum freezing rate

Frequently, the freezing process is characterized by specification of freezing rate, where in most cases it isreally the temperature change of the heat sink that is specified, or at best, the cooling rate of the solution is given. However, there are at least two freezing parameters that are needed to define the freezing process—the degree of supercooling and the rate of ice crystallization. The degree of supercooling is the difference between the equilibrium freezing point and the temperature at which ice crystals first form in the sample. The degree of supercooling determines the number of nuclei and, therefore, determines the number of ice crystals formed in the sample. A high degree of supercooling produces a large number of ice crystals, and as the total amount of water that freezes is fixed, the ice crystals produced after completion of freezing are small in size. Size of ice crystals impacts on process design and may also affect product quality, as will be discussed later. The rate of ice crystal growth determines the residence time of the product in a freeze-concentrated fluid state. A rapid growth rate minimizes the residence time, normally allowing less degradation during the freezing process. In practical freeze-drying applications, heat transfer limits the rate of ice growth. Therefore, in vials and pans where heat removal is through the container bottom, rapid ice growth is facilitated by a small fill volume to container area ratio (i.e., small fill depth) and good contact between the container bottom and the freeze dryer shelf. In general, rapid ice growth is also promoted by a low shelf temperature. However, if a warm vial with a large fill depth is placed directly on a very cold shelf, a very high degree of supercooling may be produced near the vial bottom before convective mixing cools the upper portion of the solution. Ice then forms near the vial bottom whereas the remainder of the solution remains completely liquid. Freezing then continues slowly from bottom to top as a freezing front. Such a freezing pattern normally produces a dried cake with two distinct structures: a region of very fine pores near the bottom, with most of the cake having very large pores due to the very large ice crystals that formed during advance of the freezing front. The product may be perceived as "lacking in elegance." At the least, a process designed to produce a fast freeze gives instead a slow freeze.

A high degree of supercooling produces small ice crystals and small pore size in the dried layer, which results in a high resistance to water vapor transport during primary drying.[39] Consequently, long primary drying times result. Small ice crystals also mean a high specific surface area in the dried product, which decreases secondary drying time.[40] These trends suggest that a moderate degree of supercooling is optimal. While the size of ice crystals would not normally be expected to impact on product quality, it is clear that if a protein were to denature at the aqueous ice interface, small ice crystals would mean a large interfacial area and more denaturation, perhaps leading to increased aggregation during freezing. There is now clear evidence that, at least in some cases, proteins may indeed denature at the aqueous–ice interface.[41,42]

In general, the optimum freezing process produces moderate and uniform supercooling and fast ice growth. Uniformity within a given vial avoids heterogeneous cake structure, and uniformity between vials minimizes variation in drying rates and sample temperatures. The combination of moderate supercooling and fast ice growth is difficult to achieve as, in usual practice, the cooling rate of the heat sink (i.e., the shelf) is the only controllable factor in freezing. A general procedure that normally gives satisfactory results may be summarized as follows. First, the product load is cooled to a temperature below the equilibrium

freezing point but above the temperature where experience has demonstrated that ice nucleation does not occur (i.e., typically ≈−5°C), and the load is equilibrated at this temperature for a short time (i.e., 15–30 min). Next, the shelf temperature is decreased quickly toward the final temperature (i.e., to −40°C at ≈1°C/min). At least with a small fill depth (≈1 cm), ice nucleation usually occurs uniformly with moderate supercooling, yet ice growth proceeds relatively rapidly. When product temperature is monitored with temperature sensors in selected vials, the time at which all measured product temperatures are below a given temperature (i.e., time when the product is several degrees above the final shelf temperature) is recorded. Since vials containing temperature sensors often freeze sooner than the batch as a whole,[43] the product load is allowed to soak for a fixed time (about an hour) to allow all vials to "catch up" and reach the desired temperature at which all product is in the solid state.

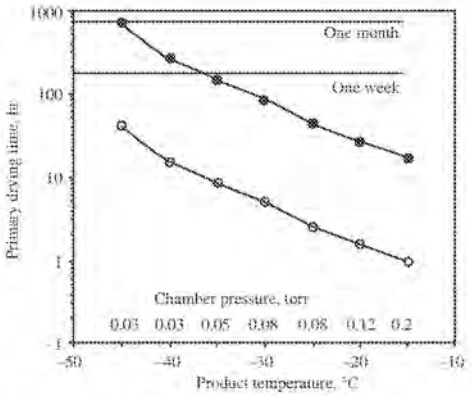


Fig. 3 Calculated primary drying times for a typical product as a function of product temperature. Open circles = 0.5-cm fill depth; filled circles = 2.0-cm fill depth.

Primary Drying

Mass transfer resistance, product temperature, and drying rate

The effect of product temperature and mass transfer resistance on sublimation rate may be mathematically illustrated[39,44] by expressing the sublimation rate per vial, dm/dt, in terms of the driving force for transport of water vapor from the ice–vapor interface to the chamber, $P_0 - P_c$.

$$\frac{dm}{dt} = \frac{P_0 - P_c}{R_p + R_s} \quad (1)$$

where P_0 is the equilibrium vapor pressure of ice at the temperature of the frozen product, P_c is the pressure in the drying chamber, R_p is the resistance of the dried product layer, and R_s is the stopper resistance. As P_0 increases exponentially with temperature, it is obvious that the driving force for sublimation, and, therefore, also the sublimation rate, increases dramatically as the product temperature increases (about a factor of two per 5°C increase in temperature). The decrease in primary drying time with increasing product temperature is illustrated by Fig. 3. These data represent calculations based upon integration of Eq. (1) for a hypothetical (but typical) product at both a small (0.5 cm) and large (2.0 cm) fill depths. The value of chamber pressure used varies due to an attempt to maintain the condition P_0 P_c, but yet employ chamber pressures near the optimum range for uniform heat transfer (≈0.1–0.2 Torr) when possible.[44] At the lowest product temperatures, the chamber pressures selected are well below the optimum for uniform heat transfer, but much higher pressures would give very long drying times. At each temperature, drying time is roughly proportional to the square of the fill depth, and target temperatures below −40°C become impractical for 2-cm fill depths.

It should be noted that Eq. (1) assumes that the gas in the vial is essentially 100% water vapor. Both theoretical and experimental evidence indicate that, during most primary drying conditions of practical interest, the gas in the vial and the gas in the drying chamber is nearly all water vapor, even when the chamber pressure is being controlled by an inert gas leak.[44] The molar flow rate of inert gas (i.e., N_2) leaked into the drying chamber is generally much smaller than the molar flow rate of water vapor during primary drying, so the composition of gas in the drying chamber remains mostly water vapor. The inert gas "overwhelms" the vacuum pumps and, therefore, increases the total pressure in pumping system, which then causes a "back-up" of water vapor into the drying chamber.

Clearly, fast freeze-drying demands both high target product temperature and a small fill depth, which unfortunately is not always possible. To maintain product elegance, and in some cases to minimize degradation, primary drying must be carried out at product temperatures below the collapse temperature. As noted earlier, the collapse temperature and T_g' are closely related, with the collapse temperature normally being several degrees higher than T_g'. In practice, recognizing that not all vials freeze dry at exactly the same temperature, the target product temperature is chosen several degrees below the collapse temperature to provide a safety margin. A collapse temperature is a property of all components in the amorphous phase,

and consequently the collapse temperature is highly formulation dependent.[45,46]

Although the product temperature is generally the most important factor in determining the rate of primary drying, product resistance is also an important parameter. The dried product resistance, R_p depends on the cross-sectional area of the product, A_p, by the relation $R_p = \tilde{R}_p/A_p$, where $\tilde{R}_p$ is the area normalized product resistance, which is independent of the sample area but depends on both the nature of the product and the thickness of the dried product. Thus, R_p depends on the container used in that A_p is fixed by the internal diameter of the vial. The units used for $\tilde{R}_p$[39,44] are cm^2 Torr hr g^{-1}. With this choice of units, the numerical value of $\tilde{R}_p$ represents the approximate time (in hours) to freeze dry a 1–2 cm thick sample at a temperature of −20°C, if the resistance would remain constant over the duration of primary drying. As one might expect, the resistance increases as the dry layer increases in thickness, and therefore, resistance increases as primary drying proceeds. However, the relationship between resistance and thickness is not usually a direct proportion, although the resistance is often roughly linear in dry layer thickness.[39,44]

Variation of product resistance with concentration is illustrated by the data in Fig. 4, where values of the mean product resistance, $\langle R_p \rangle$, are given for various product types at different total solute concentrations. The mean product resistance is the mean resistance over a dry layer thickness interval of 0–1 cm. The scatter reflects formulation-specific effects whereas the general trend with increasing concentration illustrates the tendency of higher concentrations to produce higher resistance and therefore, longer drying times. A typical value for the resistance of a small 13-mm finish stopper is shown as a straight line. Note that except for very dilute solutions, $R_p \gg R_s$.

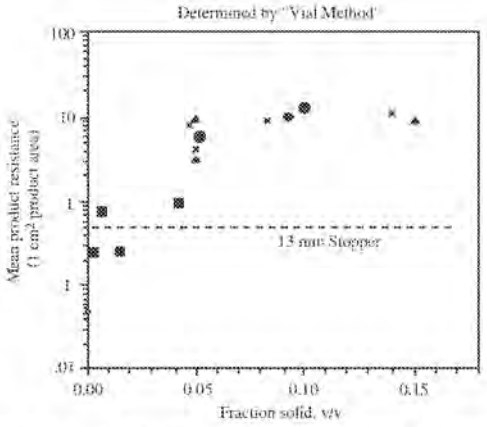


Fig. 4 Mean dry product resistance values for various products. Squares = proteins; filled circles = peptides; triangles = carbohydrates; open circle = pure ice; crosses (x) = miscellaneous types. (M. J. Pikal, Eli Lilly & Co., unpublished results.)

Process control: product temperature measurement

In most commercial freeze drying processes, chamber pressure, shelf temperature, and time are the only controllable process parameters. Product temperature is not directly controlled. It is the balance between heat and mass transfer that determines the product temperature.[47] Obviously, shelf temperature is important in determining the heat transfer and product temperature. However, because much of the heat is transferred through the gas phase (i.e., collisions of gas molecules with the hot shelf surface and the cold vial bottom), heat transfer as well as mass transfer Eq. (1) is determined, in part, by the chamber pressure. Therefore, product temperature is determined by shelf temperature, chamber pressure, the heat transfer characteristics of the vials, and the mass transfer characteristics of the product and semistoppered vials.

Conventionally, product temperature is monitored in a small number of vials by placing temperature sensors at the bottom center of the vials. It is assumed that at least the average of the measured temperatures is representative of the rest of the vials. The trend in product temperature with time is often used to determine the end of primary drying. That is, when the measured product temperature shows a sharp increase near the anticipated end of primary drying, and begins to approach the shelf temperature, it is assumed that all ice in that vial has been removed. Generally, for that vial, this assumption is correct. When all vials containing temperature sensors are judged dry by this criterion, one might be tempted to assume that all vials in the batch are dry. This assumption is not generally correct. Vials containing temperature sensors are not representative of the batch as a whole. There exists a temperature and drying rate bias between the monitored vials and the rest of the batch. Monitored vials usually freeze sooner with less supercooling than does the batch as a whole.[43] This effect is particularly significant in production where due to the particle-free environment, the introduction of a temperature sensor introduces a significantly higher level of heterogeneous nucleation sites, thereby causing nucleation of ice at a lower temperature than in vials without temperature sensors. Observations made during a production run on moxalactam are illustrated in Fig. 5.[43] Monitored vials supercool to around −15°C and then begin freezing. The vials not monitored do not begin freezing until

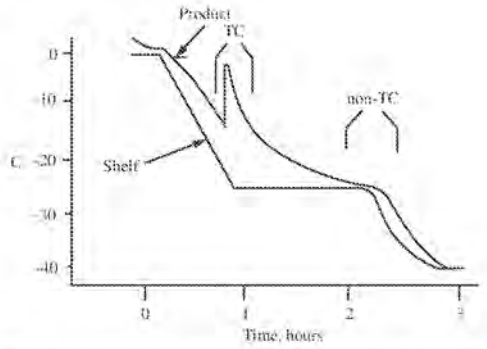


Fig. 5 Experimental observation of freezing bias: a production run of moxalactam di-sodium (from Ref.[42].)

much later, at which time the product temperature must be close to the shelf temperature of −25°C. Thus, non-monitored vials undergo a much greater degree of supercooling, have smaller ice crystals, smaller pores, more resistance to mass transfer, freeze dry at higher temperature, and require more time to dry than the rest of the vials. The temperature bias during primary drying is usually small, ≈1°C, but the drying time bias is 10% of the primary drying time, which can be quite significant for long primary drying times.[43] To compensate for this bias in drying time, a soak period of 10–15% of the primary drying time is imposed following the time when all monitored vials are judged dry. Only after this soak period is the shelf temperature increased for secondary drying. Premature increase of the shelf temperature carries a high risk of collapse. Thus, although monitoring the temperature of the product in vials does provide some information on the progress of primary drying, the information provided is far from perfect!

Location of the monitored vials in the vial array on the shelf is also an important factor in recording representative data. Vials on the edge of an array (i.e., facing the dryer door or walls) normally are not representative of the vials in the interior of an array, which are surrounded by other vials.[44] Due to differences in radiative heat transfer, such vials normally dry faster at a higher temperature. With a shelf temperature around 5°C, temperature bias is about 1°C, with drying time bias about 10%,[44] and the effect increases in magnitude as the difference between shelf temperature and ambient temperature increases and chamber pressure decreases. However, to minimize the risk of loss of sterility in surrounding vials, the temperature sensors are frequently placed in vials on the edge of the vial array facing the door. Again, the monitored vials are not representative of the batch as a whole. Clearly, minimizing the risk of product contamination with microorganisms is extremely important. From a sterility assurance viewpoint, the ultimate low-risk process is achieved with fully automatic loading systems, which, in principle, can eliminate the need for human presence in the sterile block except in emergency situations. However, it is difficult—perhaps impossible—to place temperature sensors in product vials when using an automatic loading system. Given the problems noted above with the routine use of product temperature sensors, it would seem reasonable to employ product temperature sensors only in development and validation, where vials near the center of an array could be monitored. With a robust process and suitable validation data, it may be assumed that production batches would run with the same product temperature history as the development and validation batches. Thus, product temperature would not be monitored during routine manufacturing. The simplest process design involves using a fixed shelf temperature : chamber pressure : time program.

Designing an *efficient* and *robust* process based upon a fixed shelf temperature : chamber pressure : time program is not a difficult assignment when the primary drying time is short. For example, if the primary drying time of an average batch is 6 h, designing for worst case of perhaps twice the primary drying time of an average batch would extend the average process time by only 6 h; however, if the primary drying time of an average batch is 4 days, designing for worst case will result in a significant reduction of production capacity. By using methodology for remote sensing of the end point of primary drying,[43] which will be discussed later, this process inefficiency can be virtually eliminated. Moreover, it is both possible and practical to monitor product temperature without placing temperature sensors in the product vials.[48] This methodology, called manometric temperature measurement, is based on an analysis of the rate of pressure increase in the chamber when the valve separating the drying chamber from the condenser chamber is periodically quickly closed (and the pressure control system is deactivated) for a brief period of time (≈15 s). When the drying chamber is thus isolated from the condenser chamber, the chamber pressure increases due to four effects (Fig. 6): (1) sublimation at the ice–vapor interface at constant product temperature and transfer of water vapor through the dried cake; (2) dissipation of the temperature gradient in the frozen layer, thereby increasing the temperature at the ice-vapor interface; (3) heat flow from the shelf to the product, thereby increasing the temperature at the ice–vapor interface; and (4) natural air leaks from the surroundings into the chamber. The pressure rise data is fitted to a theoretical function to obtain the average product temperature at the ice–vapor interface. Effects (3) and (4)

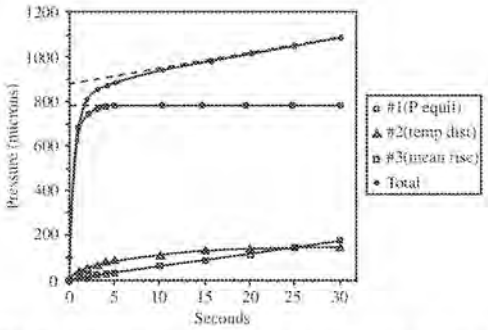


Fig. 6 Calculated contributions to the pressure rise in a manometric temperature measurement experiment. Calculations were made for a typical product with an initial ice temperature of −20°C, corresponding to an initial vapor pressure of 775 m Torr. Open circles = effect 1, sublimation; open triangles = effect 2, dissipation of temperature gradient; open squares = effect 3, heat flow from shelf to product; filled circles = sum of all effects. (Adapted from Ref.[48].)

both produce a linear increase in pressure with time whereas the other two effects are non-linear. Effect (1) is dominant (Fig. 6), but a consideration of the other effects is necessary to obtain accurate product temperatures.[48] Manometric temperature measurement gives the temperature at the ice–vapor interface, which is the temperature relevant to collapse, and most important, gives a representative temperature of the product vials without risk of sterility compromise. The freeze dryer, however, must be computer-controlled so that product temperature data can be obtained in real time via mathematical analysis of the pressure rise data. Manometric temperature measurement can also be used to sense the end point of primary drying as well as providing accurate dried layer resistance data.[48]

Process control and chamber pressure

Pressure control may be accomplished by one of three methods: controlled nitrogen leak, conductance control, or condenser temperature control. Pressure control by "controlled nitrogen leak" is accomplished by opening/closing a needle valve connected to a nitrogen source at atmospheric pressure in response to the deviation of the measured chamber pressure from the set point. Very fine pressure control, within ±5 m Torr, ily be achieved. Typically, sterile nitrogen is leaked into the drying chamber, but equally fine pressure control is achieved by leaking nitrogen into the vacuum line near the vacuum pumps. Although, to my knowledge, no relevant data exists, current dogma suggests that leaking into the drying chamber is preferred because the risk of sterility compromise is less. Conductance control is based upon modulating the resistance of the chamber to condenser pathway by partially closing/opening the valve separating the drying chamber from the condenser chamber.[49] One disadvantage of this technique is the inability to control pressure during secondary drying. Once evolution of water vapor slows to very low rates (during secondary drying), the chamber pressure will reduce to whatever ultimate vacuum the system will produce that day. Although control of chamber pressure to regulate heat input is only necessary in primary drying, one could argue that a process that does not control pressure throughout the process is not fully reproducible. In general, this argument is without scientific foundation, but there are special circumstances where product contamination by adsorption of volatile stopper impurities (or other foreign vapors in the freeze dryer) may occur, and such contamination is more serious at very low chamber pressures.[50] Here, one would want to control chamber pressure in secondary drying at a somewhat elevated level (≈200 m Torr). Chamber pressure can also be controlled by control of condenser temperature, provided the freeze dryer design provides for fine control of condenser temperature.[49] Control of condenser temperature controls the vapor pressure of ice on the condenser, thereby controlling the partial pressure of water in the drying chamber. Due to natural air leaks and the finite pressure difference between condenser and chamber required for mass transfer, the controlled chamber pressure will be somewhat higher than the vapor pressure of ice on the condenser, perhaps 20–50 m Torr higher. Thus, to control chamber pressure at 200 m Torr, the condenser temperature would be controlled at a temperature around −35°C. With this mode of pressure control, the vapor in the drying chamber remains essentially 100% water vapor throughout the process, including secondary drying. Conversely, with pressure control by a nitrogen leak, the vapor changes from essentially 100% water vapor in primary drying to mostly nitrogen during secondary drying (Fig. 7). Thus, a process run with a nitrogen leak to provide pressure control is not necessarily the same as the corresponding process run with pressure control via control of condenser temperature. In general, the difference is not expected to be of practical significance. With a final product temperature of 25°C or greater in secondary drying, a chamber pressure of 200 m Torr pure water vapor produces a relative humidity of less than 0.8%, which is not high enough to impede secondary drying of most materials. However, chamber pressure control via condenser temperature control will not produce the change in gas composition noted in Fig. 7, as the system passes from primary drying to secondary drying, which does limit the process control options for determining the end point of primary drying.

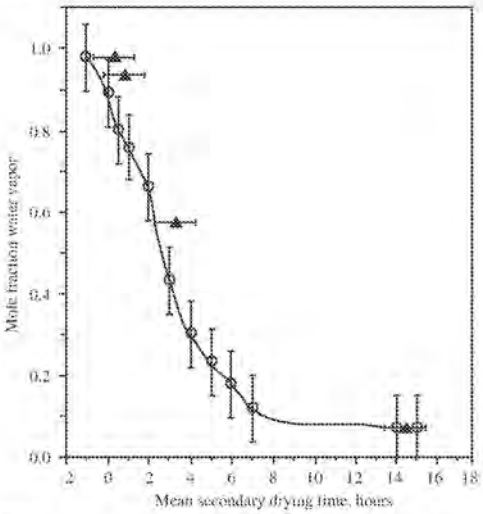


Fig. 7 Typical variation in gas composition in the drying chamber during secondary drying. Open circles: experimental values determined in a laboratory freeze dryer; filled triangles: theoretical values calculated from mass transfer theory and freeze dryer characteristics. (M.J. Pikal, Eli Lilly & Co., unpublished results.)

Process control: effect of condenser performance

Depending upon dryer design and operating conditions, the condenser temperature may vary over a wide range. As long as the condenser temperature remains sufficiently low to allow control of the chamber pressure at the desired set point, the temperature of the condenser has no impact on the freeze drying process [i.e., no change in any of the variables in Eq. (1)]. For example, if the chamber pressure is being controlled (via a nitrogen leak) at the target pressure of 0.10 Torr with a condenser temperature of −50°C, reduction of the condenser temperature to −70°C will have no effect on the process. Here, the reduced partial pressure of water at the condenser will be compensated by an increased partial pressure of nitrogen arising from an (automatic) increase in nitrogen leak rate. Although a detailed analysis of condenser performance is complex[49] and beyond the scope of this chapter, it should be noted that under conditions of very high sublimation rate, the condenser system may be overloaded, resulting in loss of pressure control (i.e., the chamber pressure increases beyond control). Unless the shelf temperature is sharply decreased, a "runaway" condition may develop with loss of product temperature control and ultimately, loss of the batch due to product collapse or ice melt. An overloaded condenser may reflect uneven ice build-up at the condenser plates caused by suboptimal design or operation, but may also arise from operation beyond the design capability of the refrigeration system.[49] That is, the refrigeration system may not be able to remove heat from rapidly condensing water vapor (i.e., from very high sublimation rate) and yet maintain the condenser plate temperature low.

Process control: determination of the end point of primary drying

Typically the shelf temperature setting used in primary drying is much lower than the shelf temperature required for efficient removal of residual water during secondary drying. Because an increase in shelf temperature before all vials have completed primary drying carries a high risk of product collapse, some indicator of the end of primary drying is needed for optimum process control. Of course, one can increase the temperature for secondary drying at a fixed time, but as discussed earlier, such a process is not an optimum one (i.e., the process is normally longer than necessary). Product temperature response is the most common indicator of the end of primary drying. Here, the time is noted when all product temperature sensors approach the shelf temperature being used in primary drying. Next, a delay time or soak period is introduced to compensate for the fact that the vials containing temperature sensors are not typical of the batch as a whole. After this delay time, the shelf temperature is increased from the primary drying setting to the setting used for secondary drying. As determination of the appropriate delay time is difficult, the delay times used are often quite arbitrary. The principle problem is that freezing bias normally differs considerably between development and manufacturing. Thus, the optimum delay time for manufacturing cannot be determined in most development laboratories. It is obvious that use of product temperature sensors to determine the end point of primary drying is not entirely satisfactory. As suggested earlier, manometric temperature measurement may be used to determine the end point of primary drying. A far simpler method is to base the determination of the end point upon a real time measurement of the vapor composition in the freeze dryer chamber. As primary drying ends, and the process passes into secondary drying, the composition of the vapor in the drying chamber shifts from nearly pure water vapor to nearly pure nitrogen (Fig. 7), assuming chamber pressure is being controlled via a nitrogen gas leak. A sensitive and inexpensive measurement of vapor composition is provided by an electronic moisture sensor with output in dew point or partial pressure of water.[43,51] An electronic moisture sensor has the sensitivity to determine presence of residual

ice in less than 1% of the vials,[43] and under some conditions can also be employed to determine the end point of secondary drying (i.e., when the residual moisture has been reduced to below the target level).[51]

Secondary Drying: Desorption of Water from the Freeze Concentrate

Product temperature control and glass transitions

The concept that one must maintain the product temperature below the collapse temperature during primary drying is now well accepted. Exceeding the collapse temperature will cause loss of product elegance, which will vary from moderate deformation and shrinkage to a complete melt and deposition along the walls and bottom of the vial. Structural collapse also may occur during secondary drying or during storage of the dried product, particularly if the dried product contains high levels of residual moisture, and the storage temperature is high. If the product temperature exceeds its glass transition temperature, cake shrinkage and deformation will occur. Such an event is a less common problem during secondary drying than is collapse during primary drying, largely because most secondary drying processes are extremely conservative during the early stage where a glass transition is most likely. As the water content of the amorphous phase decreases during drying, the glass transition temperature increases very sharply. Some representative data are shown in Fig. 8. If the shelf temperature remains at the setting used in primary drying for the first few hours of secondary drying, as is common practice, the glass transition temperature will nearly always rise much faster than will the product temperature. However, if one were to optimize secondary drying, and therefore, eliminate most of the dead time in early secondary drying, the potential for a glass transition would increase dramatically. Here, a knowledge of the glass transition temperature as a function of water content would be required for process optimization.

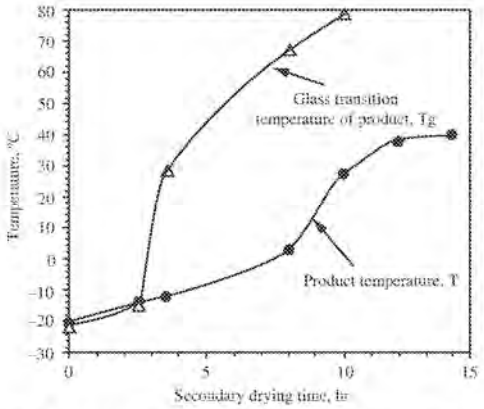


Fig. 8 Variation of glass transition temperature and product temperature for moxalactam di-sodium during secondary drying. (Calculated from data in Refs.[25,40].)

Optimum residual moisture

The optimum residual moisture for a given product must be established by empirical studies. Certainly, to eliminate the possibility of a structural collapse during storage, the water content needs to be low enough so that the glass transition temperature is well above the highest temperature relevant to product distribution and storage. Moreover, in our experience with both small molecules and proteins, in-process degradation is generally relatively insensitive to the final moisture content, and storage stability normally improves as residual moisture decreases, although the relationship between stability and residual water is not necessarily linear. Data for human growth hormone (hGH) formulated with glycine and mannitol,[19] human serum albumin,[52] and hemoglobin formulated with sucrose[53] illustrate the range of behavior normally encountered (Fig. 9). The rate of chemical degradation of hGH at 25°C (methionine oxidation and asparagine deamidation) increases in highly non-linear

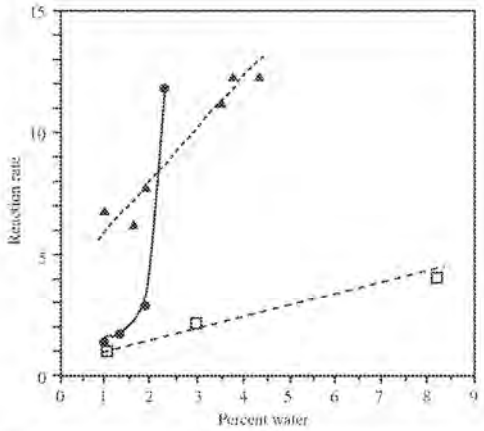


Fig. 9 The effect of residual water on the stability of freeze-dried proteins. Circles = human growth hormone deamidation and oxidation at 25°C, %/month; squares = aggregation of human serum albumin at 50°C, %/day; triangles = hemoglobin oxidation at 23°C in a sucrose formulation, %/year (From Ref.[19,52,53].)

fashion by nearly an order of magnitude as the moisture content varies from 1% to 2.5%;[19] the rate of aggregation of human serum albumin increases linearly with increasing moisture content;[52] and the rate of hemoglobin oxidation at room temperature doubles as the residual water content increases from 1% to 4%.[53] Although the optimum moisture content for storage stability may be zero, the gain in stability between about 1% water and zero water content is often not sufficient to justify the additional processing complications in achieving and maintaining extremely low water contents. An exception is storage stability of a monoclonal antibody–vinca alkaloid conjugate formulation where stability is sensitive to very low levels of residual moisture.[22]

The decrease in storage stability accompanied by an increase in residual moisture is often interpreted in terms of molecular mobility in the solid. That is, higher water content facilitates the mobility needed to support reactivity of the protein. One interpretation states that above monolayer levels of water, the protein has increased conformational flexibility, and the additional water has the ability to mobilize water and other potential reactants in the amorphous protein phase.[54] Both effects are expected to increase the rate of protein degradation. Alternatively, water plasticizes the amorphous phase, thereby lowering the glass transition temperature, T_g. Indeed, if sufficient water is present to lower T_g below the storage temperature, the amorphous phase would be in a fluid state, with greater mobility and greater reactivity than when stored below T_g in a glassy solid state. A system stored above its T_g would also suffer cake collapse and loss of pharmaceutical elegance. Although the monolayer and the glass transition interpretations are similar in that they both attribute the increased reactivity to water induced mobility in the amorphous phase, they differ in that a system above the monolayer level of water is not necessarily above the glass transition temperature. Furthermore, as the level of water equivalent to monolayer coverage is essentially independent of temperature, the monolayer concept predicts that the trend in stability with water content is independent of temperature. For example, if monolayer coverage is equivalent to 10% water, protein reactivity would increase sharply above 10% water at all temperatures. Conversely, the glass transition interpretation states that the key stability variable is the difference between the glass transition temperature and the storage temperature, $T - T_g$. Here, reactivity increases sharply when $T > T_g$, so the sudden onset of reactivity depends on both T and the water content through the effect of water content on T_g.

Although most empirical and theoretical evidence suggest that storage stability improves monotonically as the water content decreases, we must also acknowledge the common assumption that there is a critical nonzero level of water that a protein requires for conformational stability in the freeze dried solid, and therefore, some intermediate level of residual water would be optimum for stability. Although direct experimental evidence for this assumption is meager, some systems do have inferior stability when highly desiccated. Influenza virus is clearly less stable when the residual moisture deviates from the optimum 1.7%,[55] but the direct relevance of this observation to protein formulations is uncertain. Aggregation of excipient-free tissue plasminogen activator at high temperature is faster at low water content,[56] and aggregation of excipient-free hGH during storage at 25°C is faster in samples that, at some time in their history, have been highly desiccated.[19]

Factors impacting drying rate

Water removal during secondary drying involves diffusion of water in the glassy solid, evaporation at the solid–vapor boundary, and flow through the pore structure of the dried cake. During the early portion of secondary drying, water: content is high, and the drying rate is high in spite of the relatively low temperature of the solid.[40] An illustration of secondary drying kinetics under isothermal conditions is given in Fig. 10[40] for samples initially about 7% water. Mannitol is crystalline whereas povidone and

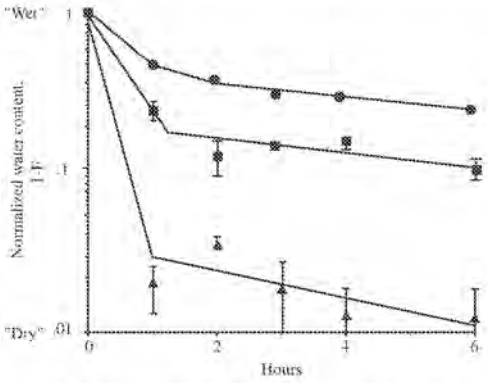


Fig. 10 Examples of the kinetics of secondary drying. Triangles = mannitol (crystalline); squares = poly (vinylpyrrolidone); circles = moxalactam di-sodium (amorphous). All solids were prepared by freeze-drying a 5% aqueous solution from a 1-cm fill depth, followed by hydration to a uniform moisture level of ≈7%. The quantity, F, is the fractional attainment of equilibrium, which corresponds to near zero water content. The secondary drying conditions were: product temperature = 18°C; chamber pressure = 200 m Torr. (From Ref.[40].)

moxalactam are 100% amorphous. Even at the start of the experiment, the glass transition temperatures of povidone and moxalactam are well above the sample temperature of 18°C. Thus, water is being removed from either an essentially crystalline solid (mannitol) or glassy amorphous solids (povidone and moxalactam). The symbol F represents the fractional attainment of equilibrium, which in these experiments is near zero water content. Thus, $1\text{-}F$ represents a normalized water content with a value of unity representing no progress in drying. Although the quantitative aspects of drying behavior are obviously specific to the product, in each case, the water content is reduced sharply during the first 1–2 h, followed by a period of much lower drying rate. If the drying rate were directly proportional to the residual water content, drying kinetics would be first order, and the curves in Fig. 10 would be straight lines. A simple diffusion model based upon constant diffusion constant and constant area for diffusion would also predict linear semilog drying curves.[40] Obviously, the curves are not straight lines. The residual water content appears to approach a plateau level, which is specific to the product. For the mannitol data, the plateau level of residual water is very low (≈0.1–0.2%) and probably represents occluded water in the crystalline mannitol. For the amorphous samples, povidone and moxalactam, the plateau levels are quite high, ≈1% for povidone and ≈3% for moxalactam (i.e., a significant fraction of the water appears to be bound). However, calculations based upon equilibrium water desorption isotherms and the measured partial pressures of water in the drying chamber demonstrate that the residual water present at the end of 6 h is far above the equilibrium water content.[40] Thus, the residual water is *not* bound in a thermodynamic sense, but rather is kinetically trapped, a result, at least in part, of decreasing effective surface area for desorption of water as the smaller more rapidly drying solid particles are dried.[40] For a given product, the plateau level of water decreases as the drying temperature is increased, decreases as the specific surface area of the solid increases, but is relatively insensitive to the thickness of the dried cake.[40]

Traditional freeze drying practice has often used very low chamber pressures during secondary drying, presumably in the belief that the rate of secondary drying would be accelerated by the use of the low pressures. However, it is found[40] that the rate of secondary drying is insensitive to chamber pressure, at least with pressures in the range of 0–0.2 Torr.[40] This empirical observation is consistent with the conclusion that the rate-limiting mass transfer process for drying an amorphous solid is either diffusion in the solid or evaporation at the solid–vapor boundary, probably the latter.[40] As the use of very low pressures in secondary drying may facilitate contamination of the product by adsorption of impurity gases from the stopper or other sources,[50] it is clear that relatively high chamber pressures (≈0.1–0.2 Torr) should generally be used for secondary drying. Of course, extremely high chamber pressures (≈1 Torr) should perhaps be avoided. With very high chamber pressures, flow of water vapor through the pore structure would become rate limiting, and the very high pressure would then decrease the rate of drying.

Changes in moisture content during storage

Often one employs a heroic secondary drying process to reduce the residual water content to very low levels only to find that the moisture content increases during storage. Although moisture transfer from ambient through the stopper is possible, the increase in moisture content is most often related to moisture release by the stopper.[57] This phenomenon is illustrated in Fig. 11, where the time dependence of moisture content in 25 mg of freeze-dried lactose is given. Here, the stoppers were gray butyl stoppers that had been previously steam sterilized, followed by 1 h of vacuum drying to remove excess water. The symbols represent experimental data for 40°C, 25°C, and 5°C storage, and the smooth curves represent the best fit to a theoretical model.[57] It should be noted that at least at 40°C and 25°C storage, the moisture content increases sharply from 1%, but then reaches a plateau value of about

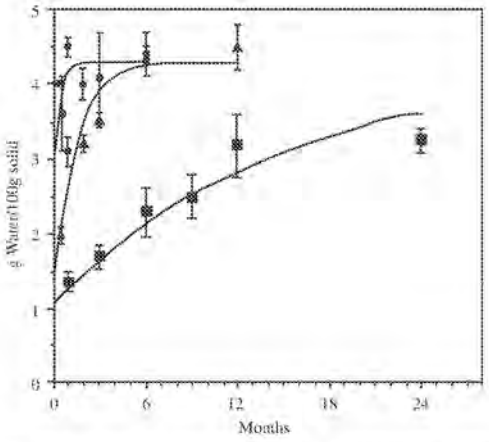


Fig. 11 Kinetics of water transfer from stoppers to 25 mg freeze dried lactose. The stoppers were 13 mm finish West 1816 gray butyl stoppers that were steam sterilized and vacuum dried for 1 hour. Circles = 40°C; triangles = 25°C; squares = 5°C. (From Ref.[57].)

4.3%, suggesting that equilibrium has been reached. That is, at the plateau value of 4.3% water, the activity of the water in the stopper is equal to the activity of water in the lactose and mass transfer ceases. In other studies, we have noted samples deliberately prepared to be initially of high water content have lost water during storage. In short, there is moisture equilibration between moisture in the stopper and moisture in the product that may either increase the moisture content of a dry product or decrease the moisture content of a wet product. The plateau or equilibrium level of water appears to be independent of temperature. The equilibrium values for 40°C storage and 25°C storage are identical, and the moisture content values for 5°C storage appear to be approaching the same equilibrium value of 4.3%. The rates of approach to equilibrium, however, is strongly temperature dependent. The equilibrium level of water is strongly dependent upon the stopper treatment history. Stoppers vacuum dried for 8 h after steam sterilization allowed only a small increase in moisture content during storage. The equilibrium water content is lower for a higher mass of solid, and is slightly higher for a more hygroscopic product.[57]

The moisture exchange between stopper and product may have important stability consequences. Absorption of moisture from the stopper may result in a product which has, after time, a moisture content high enough so that the glass transition temperature is below the storage temperature. Structural collapse will result, and stability of the product may well be compromised. Moreover, because the glass transition temperature often changes dramatically in a narrow range of water content, the onset of instability could be quite sudden. Moisture exchange may be moderated by extensive high temperature vacuum drying of the stoppers after steam sterilization. A better solution is to employ alternate rubber formulations that are less prone to release water to the product. Such stopper formulations are now available from several vendors.

FORMULATION AND STABILITY

Function of Excipients

Most freeze-dried products contain several components in addition to the drug or active component. These additional components, called excipients, are intended to serve a specific function, normally related to stability or process, and may constitute the major fraction of the freeze-dried solid.

With some products, the quantity of drug per vial is extremely small. Here, bulking agents such as mannitol or glycine are used to provide product elegance (i.e., satisfactory appearance) as well as to provide sufficient cake mechanical strength to avoid product blow-out. When a very dilute solution is freeze dried, the flow of water vapor during primary drying may generate sufficient force on the fragile cake to break the cake structure and carry some of the product out of the vial with the water vapor stream. Product blow-out normally occurs only when the solution to be freeze dried is very low in total solids (1% or less). Thus, with low dose drugs, a bulking agent may be a critical formulation component.

Collapse temperature modifiers are excipients that will increase the collapse temperature to allow more efficient freeze drying without collapse. Such materials may also function as bulking agents and/or stabilizers. Dextran ($T_c = -10°C$), hydroxyethyl starch ($T_c = -10°C$), ficoll ($T_c = -20°C$), gelatin ($T_c = -8°C$) are examples. Although none of these materials are commonly used in parenteral formulations, dextran and hydroxyethyl starch are used in large quantities as IV therapeutic agents, and therefore, would presumably be acceptable as excipients. Usually, the collapse temperature of a mixture is intermediate between the collapse temperatures of the individual components,[58] but collapse temperatures of candidate formulations cannot be predicted with high accuracy.

Macroscopic collapse may often be avoided by use of a crystalline matrix component. Here a readily crystallizable component is added at a relatively high level (i.e., more than 50% of total solids and ideally much more) such that a crystalline matrix is formed. Freeze drying such a formulation amounts to freeze drying with microscopic or partial collapse (i.e., complete collapse of the amorphous phase), but cake structure is maintained by the crystalline component. Thus, both elegance and good reconstitution time will be maintained, and the samples will normally dry to low residual water with ease. However, if collapse of the amorphous phase containing the protein leads to degradation, this method for circumventing collapse is not viable. Glycine and mannitol are commonly used as crystalline matrix components. This excipient function is, in reality, a special case of the use of a bulking agent.

Particularly with proteins, excipients are often added to prevent degradation, as for example, lyoprotectants, antioxidants, non-ionic surfactants, metal ion chelators, and other proteins such as BSA in diagnostic products. In many cases, the stabilizer may also serve as a bulking agent. Glycine, mannitol, sucrose, and lactose are perhaps the most commonly used stabilizers. However, as lactose is a reducing sugar and commonly reacts with proteins, its use must be questioned. Mannitol tends to crystallize, and is, therefore, generally a poor choice as a stabilizer. Sugars, in particular sucrose, are often effective lyoprotectants, as well as enhancers of storage stability.

Buffers are often added to control pH, but caution must be exercised when buffers are used in a formulation to be freeze dried. As discussed earlier, crystallization of either buffer component (acid or base) during freezing may cause a significant pH shift during freezing, thereby causing greater pH variation than would have been obtained in an unbuffered system.

Products intended for human use are occasionally formulated with NaCl or glycerol to make the reconstituted product isotonic. This practice is normally not a requirement for IV drugs, but can be quite important in minimizing pain on injection of subcutaneous and IM doses. In general, and in particular with proteins, isotonic adjustment is best accomplished by including the tonicity modifier in the diluent rather than in the freeze-dried product. Sodium chloride and glycerol can lower the collapse temperature significantly, and sodium chloride may cause aggregation of the protein during freeze drying.

With multidose products, there is a need for use of a preservative to prevent microbial growth during the period of product use. Mixtures of ethyl- and methylparabens are a common choice as are phenol and *m*-cresol. As preservatives are used at extremely low levels (i.e., $\leq$0.1% w/w in solution), they normally would not alter the collapse characteristics of the formulation. However, since the preservative is not needed during the freeze drying process, to keep the formulation for freeze drying as simple as possible, preservatives are best introduced via the diluent intended for reconstitution.

Surfactants may be added at low levels (i.e., $\approx$0.05% w/w in solution) for several purposes. Surfactants may aid reconstitution if the drug does wet well, and surfactants are often added to low dose products to minimize losses due to surface adsorption. Surfactants may also be effective as stabilizers in low dose protein systems.

Relationships between Formulation and Process

Particularly for freeze-dried products, formulation and process are interrelated. Properties of the formulation, in particular the collapse temperature, will have a significant impact on the ease of processing. An efficient process is one that runs a high product temperature. However, the temperature cannot be too high or product quality will be compromised. As the glass transition temperature depends on chemical composition of the amorphous phase, T_g' and collapse temperature are strongly formulation dependent. Collapse temperatures for common excipient systems vary from less than -50°C to around -10°C (Table 2).

The collapse temperature depends upon the composition of the amorphous phase, and crystallization of one or more components may significantly alter the collapse temperature. In this way, process variations that induce crystallization may alter the physical state of the formulation. For example, human growth hormone (hGH) formulated with glycine and mannitol in a hGH : glycine : mannitol weight ratio of 1 : 1 : 5 may form a completely amorphous system if frozen very quickly. Here, the collapse temperature is -24°C. However, a slower freeze allows crystallization of most of the mannitol, resulting in a collapse temperature greater than -5°C.[18] Another example is provided by the glycine : sucrose system[64–68] where glycine is present in excess of the sucrose. If frozen quickly, glycine remains amorphous, and the glycine: sucrose freeze concentrate has a very low collapse temperature (i.e., roughly -45°C, depending upon the exact composition). However, if glycine is allowed to crystallize, the glass transition temperature is essentially that of a sucrose freeze concentrate (i.e., about -34°C). Further, as the structure is maintained by the crystalline glycine, exceeding T_g' does not result in macroscopic collapse, and the system may be freeze dried without apparent collapse even at temperatures exceeding -15°C. Thus, crystallization can transform a formulation from one that is nearly impossible to freeze dry in a commercial operation to one where freeze drying is relatively easy. Clearly, if both glycine and sucrose are included in the formulation, the level of glycine must be either very low or very high relative to sucrose. If the level of glycine in the sucrose phase is very low, glycine will not crystallize but the impact on T_g' will be minimal. If the level of glycine is high, glycine can be induced to crystallize nearly completely,[66,68] thereby minimizing the level of glycine in the amorphous phase and maximizing the T_g' of the formulation.

The glass-transition temperature of a multicomponent amorphous system may be estimated from glass transition temperatures of the individual amorphous components.[69] The simplest expression, commonly referred to as the Fox equation, is

$$\frac{1}{T_g} = \frac{w_i}{T_{gi}} + \frac{w_2}{T_{g2}} \tag{2}$$

where w_i is the weight fraction of component i and T_{gi} is the glass transition temperature of pure component i. Generalization of Eq. (2) to systems of more than two components is obvious. The effect of a second solute component on a formulation may be roughly calculated from Eq. (1) if T_{gi} in Eq. (2) is identified with T_g' of aqueous component i, and w_i are weight fractions of solute relative to the total mass of solute. Although Eq. (2) does not strictly apply to a freeze concentrate containing two (or more) solute components and

Table 2 Collapse temperature T_c (°C), and glass transition, T_g' (°C) data for selected excipients[a]

Material	T_g' (°C)	References	T_c (°C)	References
Polymers				
BSA	−11	[46]		
Dextran	−10	[46,59]	−10	[60,61]
Ficoll	−19	[59]	−20	[45]
Gelatin	−9	[59]	−8	[45]
PVP (40k)	−20	[59]	−23	[45]
Saccharides and polyols				
Dextrose	−44	[59]		
Hydroxypropyl β-cyclodextrin			−18	[60]
Lactose	−28	[46,59]	−31	[60,61]
Mannitol	−35, −28	[46,59]		
Raffinose	−27	[62]	−26	[45]
Sorbitol	−46	[59]	−45	[45]
Sucrose	−32, −35	[46,63]	−34, −32	[60,61]
Trehalose	−27, −29	[46,59]	−34	[60]
Amino acids				
β-Alanine	−65	[46]		
Glycine	(−62)	[64]		
Histidine	−33	[46]		
Salts and buffer components				
Acetate, potassium	−76	[46]		
Acetate, sodium	−64	[46]		
$CaCl_2$	−95	[46]		
Citric acid	−54	[46]		
Citrate, potassium	−62	[46]		
Citrate, sodium	−41	[46]		
HEPES	−63	[46]		
$NaHCO_3$	−52	[46]		
Phosphate, KH_2PO_4	−55	[46]		
Phosphate, K_2HPO_4	−65	[46]		
Phosphate, NaH_2PO_4	−45	[46]		
Tris base	−51	[46]		
Tris HCl	−65	[46]		
Tris acetate	−54	[46]		
$ZnCl_2$	−88	[46]		

[a]Collapse temperature data were obtained with freeze drying microscopy and T_g' data were obtained by DSC at roughly 10°C/min heating rates and represent mid-points of the glass transition region. Molecular weight is given for polymers when the data are sensitive to molecular weight. When significant differences exist between laboratories, both values are given. Values in parentheses were estimated by extrapolation from non-crystallizing mixtures to the pure compound.

water, such calculations from T_g' data are sufficiently accurate to be of practical use. For example, the effect of glycine on T_g' of aqueous sucrose systems[65] is fully consistent with Eq. (2).

Bulking Agents

General considerations

Bulking agents are intended to be inert and simply function as fillers to increase the density of the product cake. Product elegance is improved and product blow-out is prevented, but the bulking agent is not intended to provide enhanced chemical or physical stability of the drug substance. Amorphous excipients can function as bulking agents, but most amorphous excipients have relatively low collapse temperatures and therefore require low drying temperatures and long processing times. For example, although lactose has been used as a bulking agent, the relatively low collapse temperature (−31°C) requires long processing times. In addition, lactose is a reducing sugar and will chemically react with amine functionality, and therefore, cannot be used with many drugs. Although not in common use as a freeze-drying excipient, hydroxyethylstarch is an example of an inert amorphous additive with a high collapse temperature and

therefore, could function as a bulking agent without requiring long processing times. However, hydroxyethylstarch tends to undergo some cake shrinkage and cracking during drying and therefore, provides a less elegant product than do the common crystalline bulking agents, glycine and mannitol. Since sorbitol is simply an isomer of mannitol, one might expect sorbitol could serve as a bulking agent. However, sorbitol does not easily crystallize during freeze-drying, and amorphous sorbitol has a collapse temperature around −45°C (Table 2), thereby preventing its use at high levels in formulations for freeze-drying. Human serum albumin (HSA) or in diagnostic products, bovine serum albumin (BSA), have seen use as bulking agents and/or stabilizers. However, given current concerns regarding excipients isolated from human sources, use of HSA in new pharmaceutical products should be questioned. Mannitol is by far the most commonly used bulking agent. A mannitol-based formulation is elegant, reconstitutes quickly, and except for the potential of vial breakage[26,70,71] is generally easy to freeze dry without risk of product defects. Vial breakage is minimized by small fill depths, lower mannitol concentration, slow freezing, and avoiding freezing temperatures less than about −25°C until crystallization is complete. Glycine functions well as a bulking agent. Glycine crystallizes easily to form an elegant product that reconstitutes quickly and does not induce vial breakage. However, a glycine cake is more fragile than a mannitol cake, and is generally perceived as being somewhat less elegant than a mannitol cake.

Polymorphism in crystalline bulking agents

In general, the polymorphism issue of greatest significance in freeze-drying is whether or not a given component is crystalline or amorphous. Which crystalline polymorph forms is usually of secondary importance. However, it should be noted that both mannitol and glycine do exhibit crystalline polymorphism in freeze-dried systems.[72–75] The only crystal polymorphism issue of known significance is the formation of a hydrate of mannitol.[75,75] Under some conditions, not fully understood, mannitol forms a hydrate that does not easily desolvate to an anhydrate during secondary drying. Secondary drying at temperatures in excess of 50°C appear to be necessary todesolvate the hydrate. Thus, samples of freeze-dried mannitol may have high residual water content if secondary drying temperatures were moderate. If this residual water were to remain bound in the hydrate crystal lattice, the high residual water content would simply be a curiosity. However, during storage, particularly at elevated temperature, the hydrate slowly desolvates, thereby releasing water to the amorphous drug phase. Such a scenario may compromise storage stability, particularly during accelerated stability tests.

Conditions for crystallization of bulking agents

A number of factors are critical for crystallization of the bulking agent. First, the nature of the bulking agent is of obvious importance. Unless the bulking agent will readily crystallize from an aqueous system at ambient temperatures, crystallization during freezing is unlikely. Although both mannitol and glycine do readily crystallize during freezing, there are many other solutes that readily crystallize and are at least potentially acceptable as excipients for parenteral drugs. Mannitol and glycine are simply the most commonly used bulking agents. Crystallization is favored by higher concentrations of crystallizable solute, and perhaps most important, the bulking agent must be the major solute component to obtain reliable crystallization. The presence of high levels of other solutes, particularly those that remain amorphous, will generally impede or prevent crystallization of the bulking agent. As a general formulation rule, one should employ the crystallizable bulking agent at a concentration (weight percent) that is at least a factor of three greater than the sum of the concentrations of all other solute components. In addition, because resistance of the dry layer to flux of water vapor increases as the concentration of total solids increases, thereby prolonging primary drying, one should avoid total solids concentrations much above 100 mg/ml when possible. A rigid adherence to both of these rules would suggest that one would not employ a bulking agent if the drug concentration were much in excess of 25 mg/ml. Indeed, because a bulking agent is normally not needed to avoid product blow-out at drug concentrations above 25 mg/ml, one should question the use of bulking agents in such applications.

Surfactants

Generally, surfactants are employed to reduce surface adsorption losses of low-dose drugs, to improve wetting and reconstitution behavior, and to stabilize proteins during freezing. In nearly all applications, it is a nonionic surfactant that is used, and by far the most commonly used surfactant is polysorbate 80. However, it should be noted that the popularity of polysorbate 80 is due more to its extensive history of use and presumed greater acceptance by regulatory agencies than to its demonstrated superior performance in a given application. In practice, the surfactant level is very low, typically 0.01–0.1% w/v.

Proteins and surface active drugs often adsorb on filters, solution-processing equipment, and container

surfaces, thereby producing loss of drug. Such losses are normally only of practical significance if the drug concentration is very low (i.e., when the total amount of drug loss is a significant percentage of the drug in solution). Addition of a low concentration of surfactant will frequently reduce the level of drug adsorption simply because the surfactant is preferentially adsorbed at the surfaces. With proteins, surface-adsorption effects may be considerably more complex and damaging. Many proteins adsorb at interfaces, particularly the air–water interface, suffer a conformational change, desorb from the interface, and either refold or combine with other conformationally altered molecules, thereby leading to irreversible aggregation.

Human growth hormone aggregates extensively during shaking an aqueous solution, but this aggregation can be nearly eliminated by adding Polysorbate 20 to the formulation.[76] It should be noted that although other surfactants (including polysorbate 80) were studied, Polysorbate 20 was the most effective stabilizer. In this study, all surfactant levels studied were above the surfactant critical micelle concentration (CMC), but the rate of aggregation decreases linearly as a function of increasing polysorbate 20 : hGH mole ratio until it exceeds 2.0. This linearity provides a clue to the stabilization mechanism. As surfactant adsorption to the air–water interface would be expected to depend on the surfactant monomer concentration, and the monomer concentration is constant above the CMC, a stabilization mechanism involving adsorption of surfactant to the air–water interface would be expected to produce a roughly constant stabilization effect at all surfactant concentrations above the CMC. The data are not consistent with this prediction. However, it is known that polysorbate 20 binds to hGH with a stoichiometry of about 2.5 to 4.[77] Thus, it seems plausible that stabilization during shaking involves binding of surfactant to the surface of the protein.[77]

Aggregation of hemoglobin during freeze–thaw is essentially eliminated by addition of polysorbate 80 at concentrations from 0.0125% to 0.1%.[78] Without polysorbate 80, particle formation after five freeze–thaw cycles increased by more than an order of magnitude when freezing to −20°C and by about a factor of five when freezing to −80°C. However, use of polysorbate 80 reduced particulate generation at both freezing temperatures to a level where particulate levels after freeze–thaw were not significantly different than before freezing. Attempts to demonstrate binding of the surfactant to hemoglobin were unsuccessful, suggesting that the stabilization mechanism does not involve binding to the protein. Rather, it was proposed that stabilization involves surfactant adsorption at the interface, thereby preventing the protein from adsorbing at interfaces.

Although one cannot always expect protein aggregation to be eliminated as effectively by addition of surfactant as demonstrated in the above examples, it is certainly prudent to test the effect of surfactants in situations where protein aggregation is a problem during freeze–thaw or during solution handling.

Stabilizers

Types of stabilizers

A stabilizer is simply a formulation component without physiological effect that is added to the formulation to maintain the physical or chemical stability of the drug substance. As discussed in the previous section, the function of a surfactant may be to stabilize, and as control of pH is often critical to stability, one might consider buffers to be stabilizers. However, in this section we will address stabilizers other than surfactants or buffers. Further, as most of the stabilization literature deals with proteins, our discussion will focus on proteins, although in principle many stabilization principles apply to small molecules as well.

Minimizing oxidation

Although oxidation is a very common degradation pathway in pharmaceutical systems, one might expect that oxidation problems in freeze-dried products would be easily solved by elimination of oxygen from the system during processing; that is, because the product is dried in a vacuum environment and the vials either sealed in vacuum or in an atmosphere of an inert gas (i.e., nitrogen), no oxygen will be present to support an oxidation reaction. Indeed, storage stability of human growth hormone is greatly improved in vials back-filled with nitrogen compared to vials back-filled with oxygen.[19] However, significant oxidation was also observed in samples back-filled with nitrogen. In practice, complete elimination of oxygen from the solution being processed is unlikely, and some oxygen may well be trapped in the amorphous solute phase after freezing and not be completely removed during drying. Further, due to a variety of causes including diffusion from the atmosphere over time, oxygen content in the vial headspace may easily reach levels of about 1%. Even 1% oxygen in the vial headspace is sufficient, from a stoichiometric viewpoint, to produce substantial decomposition, particularly with high-molecular-weight drugs. For example, with a 5-mg dose of human growth hormone (M = 22.5 kD) in a 5-cc vial, 1% oxygen in the headspace represents a factor of ten excess of the oxygen required for complete oxidation of the protein.

As ground-state molecular oxygen is not particularly reactive, presence of oxygen is not the only requirement for a rapid oxidation reaction. Activation of molecular oxygen to more reactive species requires light (i.e., photoactivation to singlet oxygen, $^1O_2$), or presence of a reducing agent and trace levels of transition metal ions (i.e., iron and/or copper), which can then convert molecular oxygen into more reactive oxidizing agents such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), or hydrogen peroxide (H_2O_2).[79] Transition metal ions are often present in excipients, and processing in stainless steel equipment can lead to significant iron contamination. As the role of the transition metal ion is catalytic, only trace levels are required. The reducing agent is consumed in the conversion of molecular oxygen into reactive oxygen species, so higher levels of reducing agent are required for significant degradation of a drug. However, higher levels could still represent contamination from impurities in the drug and/or excipients. In the example of 5 mg hGH, a low-molecular-weight reducing agent ($M \approx 100$) present at a level of 0.1% w/w of the amount of hGH could lead to oxidation of more than 10% of the protein. The reducing agent could also originate from a misguided attempt to suppress oxidation by addition of an antioxidant such as ascorbate. Ascorbate may function as an antioxidant in some circumstances but will also function as an effective prooxidant and reduce molecular oxygen to reactive oxygen species in the presence of transition metal ions such as iron or copper.[79,80] Finally, peroxides are common contaminants in polyethylene glycols and nonionic surfactants and can serve as the oxidant. Thus, at attempt to stabilize protein conformation by addition of these materials may well chemically destabilize if oxidation is a possible degradation pathway.

A number of amino acid residues are subject to oxidation. Metal-catalyzed oxidation of methionine to methionine sulfoxide and other products is perhaps the most common pathway. Oxidation of cysteine to form either nonnative intra- or intermolecular disulfide bonds is also common, and histidine residues are also easily oxidized in metal-catalyzed pathways, with tryptophan and tyrosine being degraded by light-catalyzed oxidations.[79] Mechanisms are complex, and even with a given reaction such as methionine oxidation, the active oxygen species and reaction product(s) varies with the experimental conditions;[79,80] thus, stabilization is difficult. Certainly, oxygen content in the solution being processed should be minimized, and the product should be sealed in the freeze dryer with either vacuum or nitrogen in the headspace. Due to the negative impact of even small amounts of transition metal ions, prooxidants, and peroxides, the bulk drug substance and excipients need to have rigid specifications. Formulation pH and the type of buffer salt may also be important.[80] The optimum conditions vary with the application, and some empirical screening experiments are necessary to optimize a given formulation.

Specific chemical components may also be added in an attempt to retard oxidation. The classical solution, addition of an antioxidant, must be approached with caution as the antioxidant may function as a prooxidant in a metal-catalyzed conversion of molecular oxygen into reactive oxygen species. Likewise, addition of a metal-complexing agent such as EDTA does not always retard oxidation. In fact, oxidation may be accelerated by complexing the transition metal.[80] With most metal-catalyzed oxidations, the oxidation mechanism is a site-specific one, where the reduced form of the metal binds to a residue such as histidine on the protein and converts oxygen to reactive oxygen species at that site, which in turn oxidizes another residue nearby.[81] In cases where complexation with EDTA facilitates oxidation, it is assumed that the EDTA–metal complex binds more strongly to the protein and is therefore more effective in generation of reactive oxygen species near a reactive residue.[81] However, addition of EDTA does retard oxidation in some cases,[82] and it would seem prudent to screen several complexing agents for impact on oxidation. Scavengers for reactive oxygen species, hydroxyl radical, $\cdot OH$, and singlet oxygen, $^1O_2$, may also retard oxidation,[79,83] but the results are highly specific to the system. For example, for iron-catalyzed oxidation of a methionine-containing peptide, thiourea was found effective for both ascorbate and dithiothreitol prooxidant systems but mannitol was effective only for the dithiothreitol prooxidant system.[83]

Stabilization during freezing

In the context of proteins, stability has two distinct meanings. The term pharmaceutical stability refers to the ability of a protein to be processed, distributed, and used without irreversible change in primary structure, conformation, or state of aggregation. We refer to pharmaceutical instability as degradation. However, the phrase protein stability is also commonly used to describe the position of the equilibrium between native and unfolded conformations. If a protein formulation requires a high level of chemical denaturant, or a high temperature, to shift the equilibrium between native and unfolded in favor of the unfolded state, the protein is said to be stable. This meaning of stability I call "thermodynamic stability." Thermodynamic instability involves physical changes, somewhat analogous to a thermodynamic change of state. Pharmaceutical instability may be purely a result of a physical change (i.e., non-covalent aggregation), but may also involve

changes in primary structure or, in other words, chemical degradation. Certainly, chemical transformations such as oxidation and deamidation are degradations as is aggregation to form insoluble precipitates.

Pharmaceutical stability and thermodynamic stability are not necessarily directly related. For example, a protein may exhibit thermodynamic instability during freeze-drying and unfold, but if no irreversible reactions occur during storage or during reconstitution, the reconstituted protein may refold completely within seconds and, therefore, display perfect pharmaceutical stability. A protein that remains in the native state and is thermodynamically stable may still degrade via chemical reactions such as deamidation and oxidation over storage times of years, particularly if the reactive moiety is located on the protein surface. Conversely, thermodynamic instability may well be a prelude to degradation. Certainly, an unfolded protein could expose normally buried and protected methionine and asparagine residues to the solution environment and render these residues more reactive. Also, degradation via irreversible aggregation is believed to often proceed through unfolded or partially unfolded conformations as intermediates.[84]

Many proteins survive the freeze-drying process with little or no degradation, whereas other proteins exhibit significant degradation and loss of activity during processing. Degradation during the freeze-drying process may arise during freezing and/or during drying, and it is useful to establish when in the process degradation occurs. As a measure of the degradation during freezing, freeze–thaw stability studies are carried out, and to estimate (roughly) the degradation during drying, stability during freeze-drying is compared to stability during freeze–thaw. The basic assumption is that degradation during thawing is comparable to degradation during reconstitution; therefore, the difference in activity between a freeze-dried–reconstituted sample and a freeze-thawed sample is a measure of the loss in activity during drying. This assumption is likely a reasonable approximation for a fast thawing process. One observation of particular significance is that some excipients stabilize during both freezing and drying (called lyoprotectants), whereas others stabilize only during freezing (called cryoprotectants).[41,85,86]

If degradation occurs only during freezing and practical variations in the freezing process do not eliminate the problem, it is obvious that a cryoprotectant system is required. However, as a number of distinct stresses may develop during freezing, the proper choice of cryoprotectant is not always obvious. Provided some information regarding the degradation pathway is available, the stabilization strategy could be quite specific. For example, if oxidation is suspected to be a major pathway, the recommendations regarding minimization of oxidation should be followed. If aggregation is a major problem, the use of nonionic surfactants should be considered, or if it is known that a change in pH caused by buffer crystallization is a problem, steps to minimize pH change during freezing need to be taken. Use of an alternative buffer system or simply use of very low levels of the buffer might well eliminate the pH change and solve the stability problem. Alternatively, addition of an excipient (i.e., sucrose or trehalose) that will interfere with buffer crystallization will also prevent excessive pH shift during freezing.[41,85,86]

In addition to the specific stabilization strategies noted, one may also employ a general stabilization strategy that is based upon addition of components that normally increase the thermodynamic stability of the protein. That is, addition of a component that will increase the free energy of denaturation will moderate the effect of various freezing stresses that cause a decrease in the free energy of denaturation, with the net result that even during freezing, the native conformation will remain the dominant conformation and degradation will be reduced. The assumption is that increasing thermodynamic stability will also increase pharmaceutical stability. A number of solute types (i.e., amino acids, saccharides, polyols, polyethylene glycols, and some other classes) have been found effective both in increasing thermodynamic stability and in increasing pharmaceutical stability during freezing.[85,86] These solutes are referred to as excluded solutes because such solutes are present in lower concentration near the surface of a protein. The thermodynamic consequence of such exclusion is that the protein is preferentially hydrated, and the chemical potential of the protein is increased. It may be argued that the increase in the chemical potential will be much greater for the unfolded state than for the native state, thereby increasing the free energy of denaturation and stabilizing the native conformation.[85,86] Although the increase in protein chemical potential due to solute exclusion does not always correlate quantitatively with the effectiveness of that solute as a cryoprotectant, it would be prudent to test several pharmaceutically acceptable excluded solutes (i.e., glycine, mannitol, disaccharides, polyethylene glycols) as potential cryoprotectants in cases where freezing instability might involve conformational destabilization. Obviously, polyethylene glycols would not be a good choice if oxidation were an issue. For cryoprotection, it is the concentration of the cryoprotectant in solution that is the key concentration variable, regardless of the concentration of protein in the formulation. With non-polymer excluded solutes, relatively high concentrations (i.e., 0.2–0.5 M) are normally required for effective cryoprotection.[85]

Stabilization during drying

In addition to the stresses that develop during freezing, drying imposes an additional stress associated with essentially complete removal of water. Indeed, the water substitute hypothesis[41,85–87] is based upon the proposition that a significant thermodynamic destabilization occurs when the hydrogen bonding between protein and water is lost during the last stages of drying. The use of a water substitute as a lyoprotectant allows a hydrogen-bonding interaction between protein and the water substitute, which thermodynamically stabilizes the native conformation and preserves activity. A water substitute is a moiety that is capable of hydrogen bonding to the protein surface much as water and stabilizes via a thermodynamic mechanism; that is, stabilization is achieved by maintaining the free energy of unfolding very high such that essentially all of the protein is maintained in the native conformation. However, it must be recognized that most observations can also be rationalized in terms of a purely kinetic stabilization mechanism. In general, drying is conducted at temperatures sufficiently low that the protein exists in a glassy solid state where molecular mobility is greatly restricted. It may be argued[41] that a good stabilizer is a component that effectively couples protein dynamics to the dynamics of the glass such that even if the protein is destabilized thermodynamically, motion in the protein is too slow to allow significant unfolding during the drying operation. Thus, the protein conformation is stabilized regardless of the free energy of unfolding.

Non-reducing di- and tri-saccharides such as sucrose, trehalose, or raffinose are normally good drying stabilizers.[41,85,86] They qualify as good water substitutes and also form glasses which, via hydrogen bonding, can couple protein dynamics to matrix dynamics. Under conditions of acid pH, particularly below pH 5, trehalose is a much more effective stabilizer than are sucrose or raffinose. Sucrose and raffinose hydrolyze to their reducing sugar components much more rapidly in the solid state than does trehalose, thereby leading to degradations initiated by reducing sugars.[88] The key concentration variable in drying stabilization is the weight ratio (or mole ratio) of saccharide to protein, with the weight ratio of stabilizer to protein normally being between 1:1 and 10:1. The principle here is to insure that the protein is in a matrix of the stabilizer. Thus, with dilute protein systems, good stabilization can often be obtained at rather low concentrations of stabilizer. Here, it may be desirable to employ both a bulking agent such as glycine or mannitol, as well as a stabilizer. This strategy can yield both an elegant cake structure as well as optimal stability without the high solute concentrations than can slow primary drying.

Stabilization during storage

Although the destabilization stresses during storage remain much the same as the drying stresses, the time scale of storage is obviously much longer than the timescale of freeze-drying. In addition, the freeze-dried product is normally being stored well below its glass transition temperature, meaning molecular mobility is greatly restricted.[41,85,86] Because of these differences, the dominant degradation pathway during storage may differ from the degradation pathway during freeze-drying. For example, deamidation of asparagine residues is a very common degradation pathway during storage but rarely is a problem during processing. This observation does not imply that deamidation is faster in the dry glassy solid than in solution, but rather is simply the result of the much longer time scale of storage. Indeed, because of the need to insure stability over the shelf life of about 2 years, storage stability normally presents a more serious problem than does stability during processing. Degradation during storage may arise from chemical degradation, such as oxidation and asparagine deamidation, and/or aggregation to non-covalent or covalent aggregates.[41,85,86] Aggregation often means formation of a population of very large insoluble aggregates that precipitate from solution. It must be admitted, however, that the observation that the level of aggregation increases during storage does not necessarily mean that the bimolecular process of aggregation occurs in the solid state. It is also possible that only conformational changes occur during storage. Aggregation of a fraction of the conformationally modified protein could then occur instantaneously during reconstitution. The remaining fraction would then refold tothe native conformation. As aggregation is normally measured in solution, it is not possible to determine just when the aggregation step actually does occur. When the amount of aggregation measured depends on the reconstitution procedure and/or the composition of the diluent (i.e., presence of surfactant) used to reconstitute,[32] one would conclude that, at least in part, aggregation of (partially) unfolded protein occurs during reconstitution.

Stabilization strategies focus on three general factors: (1) specific chemical requirements; (2) physical state of the solid; and (3) structure of the protein. Depending on the degradation pathway(s) and mechanisms, any one or all of these factors could be critical. Certainly, if oxidation is a major degradation pathway, stabilization requires attention to the chemical composition of the system, including both the effects of impurities and addition of stabilizers as discussed earlier. Although pH has no real meaning in the solid state, the degree of ionization in the solid does reflect the pH of the solution from which the solid was

prepared, and pH of the solution can be a major variable. Because differences in polarity of the environment between a dry solid and an aqueous solution may mean that the optimum pH for solid stability is somewhat different than the optimal pH for a solution, some solid-state screening experiments are necessary to define the precise optimum pH for stability of the freeze-dried solid.

The physical state of the system is of obvious importance. We freeze dry to increase storage stability relative to the solution state, assuming that the restricted molecular mobility of the solid state will lead to stabilization. In general, an amorphous solid (i.e., glass) is several orders of magnitude more stable than the corresponding aqueous solution, and at least with small molecules, crystalline drugs are much more stable than glasses.[89] However, proteins cannot be crystallized during freeze-drying, so the only solid-state option is a glass. Forming a glass does not insure adequate stability. Pure dried proteins are glasses and often do not have sufficient stability for long-term storage at the temperatures of interest, and excipients are added to the formulation to stabilize. Because a physical mixture of protein and excipient will not provide additional stabilization, it is necessary that the proposed stabilizer system remain at least partially amorphous and in the same phase as the protein.[41,85,86] Further, to avoid chemical reactions between the excipient and protein, the excipient system must be inert. The most common violation of inertness is the use of reducing sugars such as lactose in an attempt to stabilize the protein. For example, while lactose will stabilize hGH against aggregation, an adduct of lactose and hGH quickly forms during storage of the freeze-dried solid.[18]

Drying also removes water, a reactant in a hydrolysis reaction, and stability is normally best at very low water content.[41,85,86] However, the effect of residual moisture on stability is far more complex than its possible role as a reactant. Residual water has a major effect on the physical state of the system in that increasing levels of residual moisture decreases the glass transition temperature of the amorphous system, thereby making the system less solid-like at a given temperature, and eventually causing the glass to transform into a fluid at the storage temperature. Fig. 12 illustrates the effect of residual moisture on the glass transition temperature of two common stabilizers, sucrose and trehalose.[90] For both examples, small amounts of residual water greatly depress the glass transition temperature, but since dry sucrose has a much lower glass transition temperature than does dry trehalose, the water content that gives a glass transition temperature at room temperature, W_g (25°C), is much lower for sucrose. Thus, in applications where maintaining very low water content is difficult, trehalose is a better choice for a stabilizer than is sucrose.

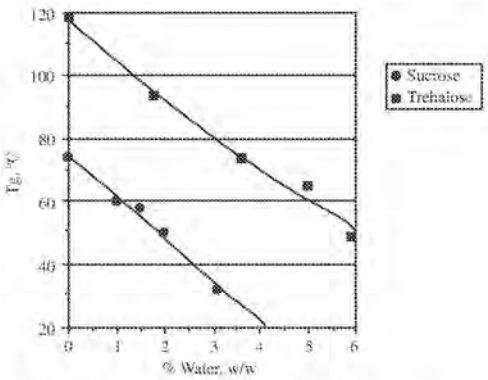


Fig. 12 The effect of residual water on the glass transition temperatures of sucrose and trehalose. (From Ref.[90].)

Generally, chemical and physical stability decrease sharply as the system enters the fluid state.[41,86,91,92] Chemical degradation of hGH in a trehalose formulation illustrates this behavior.[92] The trehalose formulation shows a well-defined glass transition temperature with DSC, with the expected decrease in T_g as the water contentis increased. The rate constant for chemical degradation is essentially independent of water content while the system remains glassy, but at least for the 50°C data, degradation increases sharply as increasing water content depresses the system glass transition temperature significantly below the storage temperature (Fig. 13). Rates of aggregation show essentially the same behavior.[92] However, as stability is not sensitive to water content in the glassy state, stability is not correlated with $T-T_g$ below the glass transition temperature. The study of formulation effects on hGH stability suggests the same conclusion. Chemical and aggregation stability of hGH in several formulations is compared in Fig. 14.[92] The glycine: mannitol formulation is a 1:1:5 weight ratio of hGH:glycine:mannitol, where only the mannitol is crystalline. The other formulations are 100% amorphous. Hydroxyethyl starch (HES), stachyose, and trehalose are formulated in a 1:1 weight ratio of excipient:hGH, whereas the dextran formulation is 6:1 dextran:hGH. While the concept that an excipient system must remain at least partially amorphous to improve protein stability is not in question, it is clear that remaining amorphous is not a sufficient condition for stability. Apparent aggregation in the dextran formulation is greater than in the pure protein. Hydroxyethyl starch shows a slight improvement in stability over the pure protein, but is not nearly as effective as the glycine:mannitol formulation, and increasing the level of HES to 3:1 HES:hGH does not improve stability.[92] Conversely, both stachyose

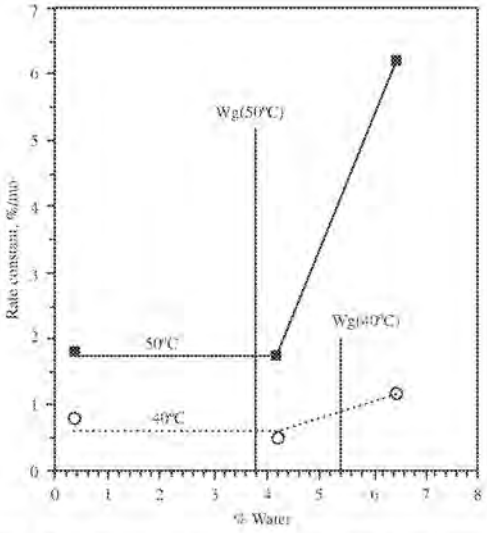


Fig. 13 Chemical degradation in freeze dried hGH formulated with trehalose as a function of water content at 40°C and 50°C. The pseudo first-order rate constant for degradation (%/month) is given for the combination of asparagine deamidation and methionine oxidation. The formulation is hGH: trehalose in a 1 : 6 weight ratio with sodium phosphate buffer (pH 7.4) at 15% of the hGH content. The highest moisture content samples were collapsed after storage at both 40°C (moderate collapse) and 50°C (severe collapse). The water content that reduces the glass transition temperature of the formulation to the storage temperature is denoted "Wg." ○ = 40°C storage; ■ = 50°C storage. (From Ref.[86].)

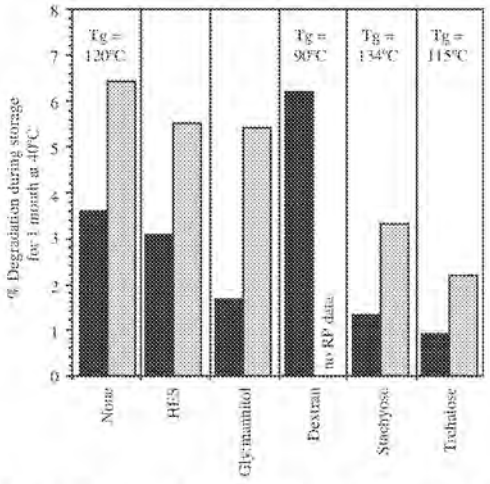


Fig. 14 The effect of excipients on the storage stability of freeze-dried human growth hormone (hGH). Samples were stored for 1 month at 40°C. Solid bars: aggregation (primarily dimer), shaded bar = chemical degradation via methionine oxidation and asparagine deamidation. The glass transition temperatures of the initial freeze-dried formulations are given above the bars when a glass transition temperature could be measured by DSC. The glycine : mannitol formulation is a weight ratio of hGH : glycine : mannitol of 1 : 1 : 5, the dextran formulation is 1 : 6 hGH : dextran 40, none means no stabilizer, and the others are 1 : 1 hGH:stabilizer. All formulations contain sodium phosphate buffer (pH 7.4) at 15% of the hGH content. Initial moisture contents are all ≈1%. (From Ref.[86].)

and trehalose provide better stability than the glycine : mannitol system does, with trehalose superior to stachyose. All systems are glasses at the storage temperature of 40°C, and for those formulations where glass transition temperatures are available, it is clear that storage is well below the T_g, and there is no simple relationship between T_g and stability. Although one might speculate that a glass is more solid, and therefore more stable, the larger the difference between T_g and the storage temperature, the data are not consistent with this speculation. Comparing the stachyose and trehalose formulations, which are both 1 : 1 formulations with hGH, the T_g of the stachyose formulation is nearly 20°C higher than that of the trehalose formulation, but trehalose offers slightly better stability than does stachyose. These observations and other similar results[92] suggest that while it is necessary for the formulation to have a T_g well above the highest anticipated storage temperature for both elegance and stability reasons, stability well below T_g is not directly related to the precise difference between storage temperature and T_g.

The lack of a clear correlation between stability and T_g for systems well below T_g is not entirely unexpected. Molecular mobility slows greatly below T_g, but does not approach zero until some temperature much lower than T_g.[41,86] Thus, dynamics in the glass depend not only on the value of T_g but are also extremely sensitive to other characteristics of the glass as well as the thermal history of the glass.[41,93] Even if the major component of the glassy matrix (i.e., the sucrose) were to have essentially zero mobility (i.e., negligible translational and rotational motion), mobility of small molecules (i.e., diffusion of water) could still be significant, and mobility of potentially reactive groups on the protein (i.e., asparagine side chain) could be sufficient to reorient into the transition state and react.[41] Thus, it is not only necessary that the matrix itself be solid-like, but effective coupling between matrix mobility and the mobility critical for degradation is needed for solid-like stability behavior.[41] Although the molecular

characteristics required for effective coupling are not fully understood, it does seem that hydrogen bonding between protein and stabilizer provides one coupling mechanism.[41,86] Thus, disaccharides and trisaccharides are effective stabilizers.

With proteins, structure is also a critical stability variable. It is now common knowledge that proteins often suffer significant conformational changes on freeze-drying that may be moderated by addition of stabilizers to the formulation.[41,85,86] In principle, different conformations may have different stability characteristics with the native conformation normally believed to represent the most stable conformation.[41,85,86] Indeed data for rIL-2 show a strong correlation between storage stability and structure as measured by Fourier transform infrared spectroscopy (FTIR),[21] with a more native conformation associated with greater storage stability. Likewise, formulations of freeze-dried hGH having more native-like conformations are more stable to both aggregation and chemical degradation during storage.[92] Thus, degradation of a protein in any given formulation is a function of the distribution and reactivites of the protein substates created during the freeze-drying process, with greater stability being associated with the more native-like substates. From this viewpoint, a good stabilizer is one that maximizes the population of native-like substates. Empirical evidence suggests that disaccharides perform this function quite well, regardless of what mechanism might be postulated to explain the observation.[41,85,86]

In summary, guidelines for stabilization during storage involve the following principles: 1) optimize pH and address specific chemical effects such as oxidation; 2) disperse the protein in an inert glassy matrix with strong coupling between protein dynamics and matrix dynamics to form an amorphous phase such that at all residual water contents and storage temperatures of interest the protein phase will be well below its glass-transition temperature (note that this requires both protein and stabilizer exist in the same glassy phase); 3) employ a formulation (and process) such that the dried protein will retain the native conformation.

Retention of native conformation, coupling of protein mobility with matrix mobility, and formation of a single phase with the stabilizer all require an excipient that interacts, probably via hydrogen bonding, with the surface of the protein. Little or no interaction would likely lead to phase separation, poor coupling of protein mobility with matrix mobility, and no opportunity for the excipient to stabilize the native conformation by either water substitution or immobilization of the protein. Disaccharides (i.e., sucrose and trehalose) and trisaccharides (i.e., raffinose) seem to satisfy these criteria and are generally good stabilizers. Polymers are generally much less effective. A product glass-transition temperature well above all anticipated storage conditions is an important product quality attribute. Glass transition temperatures of selected amorphous excipients are given in Table 3. Both trehalose and raffinose have much higher glass transition temperatures than does sucrose, and in this sense, would be better choices for stabilizers. However, as long as the residual water content is maintained low and the product is not plasticized by other low-molecular-weight formulation components, a sucrose formulation will be well below its glass-transition temperature at all practical storage temperatures. Due to a very low T_g, sorbitol should not be used as a major formulation component. Incomplete crystallization of glycine or mannitol may also lead to low product glass-transition temperatures. Lactose and maltose have high glass transition temperatures but are reducing sugars and, therefore, are poor stabilizer candidates. As noted earlier, applications at low pH pose problems for both sucrose and raffinose due to rapid hydrolysis to the reducing sugar components. Trehalose is a better choice for low pH applications.

As with drying stabilization, stabilization of a protein for storage with saccharides generally requires a weight ratio of stabilizer to protein between 1 : 1 and 10 : 1, with better stabilization at the higher excipient levels. At least for hGH, the rate constants for degradation (chemical degradation and aggregation) decrease linearly on a plot of the logarithm of rate constant as a function of stabilizer : hGH weight ratio.[92] With high dose products where the protein concentration in the fill solution is high, use of high ratios of stabilizer to protein may not feasible due to the extremely high concentration of stabilizer that would be required.

Table 3 Glass transition temperatures, (T_g), of selected excipients measured by differential scanning calorimetry[a]

Compound	T_g (°C)	References
Citric acid	11	[94]
Glycine	(≈30)[b]	[66]
Lactose	114	[95]
Maltose	100	[95]
Mannitol	13	[74,96]
Raffinose	114	[95]
Sorbitol	−1.6	[96]
Sucrose	75	[95]
Trehalose	118	[95]
Maltodextrin 860	169	[95]
PVP K90	176	[95]

[a]Consult the references for details of the techniques.
[b]Value in parentheses is extrapolated from mixtures using the Fox equation and is highly approximate.

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Freeze Drying, Scale-Up Considerations

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INTRODUCTION

Product presentation, formulation, and processing are integral in their influences on the outcome of manufacturing a lyophilized product. Activities in development involve knowledge of the clinical and stability requirements, formulation and product design, and investigation into suitable processing conditions. These activities incorporate the awareness that the product is expected to be integrated into large-scale routine manufacturing of commercial product. Multiple steps and increasing batch sizes are often necessary as the product progresses through clinical studies and market introductory batches. Aspects of equipment, processing capabilities, processing influences, and finished product attributes all need to be considered in the scale-up to increasingly larger batch sizes on the pathway of taking a product to market.

DEVELOPMENT AND SCALE-UP PATHWAY

In the progression of bringing a new product to the market, scale-up from the initial development batches to commercial manufacturing is an important activity. This progression includes transferring the technology for producing a product after sufficient initial development, eventually integrating the procedures into a commercial manufacturing environment. As part of later development activities, materials for stability and early clinical studies are commonly prepared on a small scale. This comprises the first transfer of the processing methods into a manufacturing environment. Following successful clinical results, the product presentation and any refinements to the manufacturing procedures are implemented, and the process is duplicated on a larger scale in a manufacturing environment for full commercial production.

With an approved investigational new drug (IND), a new drug entity is developed into a new product with administration to human patients for establishing safety, efficacy, and dosing regime in phase I through phase III clinical studies. Initial development activities focus on preparing materials for conducting these studies. Meeting the needs of supplying the clinical material often results in initial product presentations that may be different than the final product that is introduced to the market. As clinical trials progress, active pharmaceutical ingredient (API) manufacturing, analytical methods, product design, and manufacturing procedures are developed and refined. With the success of early clinical trials and entering into phase III clinical studies, product preparation and specifications are finalized. The API is well characterized, analytical methods are validated, product presentation and specifications finalized, as well as finished product processing procedures established.

Initial development activities are focused on preparing to supply early clinical requirements and product needs in designing the product formulation and presentation. The product presentation is based upon optimal administration to the patient in a clinical setting and stability requirements for the active ingredient. Lyophilization is used as a method of preservation for products that are sensitive to the presence of water and have limited stability as a liquid ready-to-use preparation. This low temperature vacuum drying process removes the water, leaving a dried solid, resulting in sufficient stability to allow long-term storage. At the time of use, the product is reconstituted with a diluent that returns all the attributes of a liquid ready-to-use product, yielding the preparation suitable for administration. One advantage to a lyophilized preparation is the different concentrations of API that may be obtained by varying the diluent volume for reconstitution, along with the volume used for administration, to allow for different dosages from a single preparation. Once the initial product presentation suitable for the early clinical studies is identified, processing procedures can then be developed. The focus for the initial lyophilization process is robustness and ease of scale-up rather than optimal parameters for routine manufacturing. In practice, the presentation initially developed may not necessarily be the final product marketed; the product presentation may change as a result of clinical studies. Continued development activities often then encompass a series of refinements to the product and procedures. In the pathway of bringing a new product to the market, scale-up and technology transfer may therefore occur multiple times.

As the product progresses through stages of development and clinical studies, product and process design are refined. Processing experience is gained on

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increasingly larger scale batches as the demands for clinical material increases. Finally, phase III clinical studies require product quantities that may approach batch sizes closer to those that are typical of full-scale commercial manufacturing. Fig. 1 describes objectives and environment in the progression of steps in taking a product from initial development to manufacturing-scale operations. Initial success along with expanded indications throughout the life cycle of a product may continue to require larger batch sizes to meet increasing market demand. With the evolution of the product from development to clinical studies, followed by initial introduction and potential growth with market expansion, scale-up begins with smaller and progresses to increasingly larger batch sizes.

Design of a preparation and development of a suitable lyophilization process is first conducted in the laboratory. Product design focuses on the formulation and presentation. Stabilizing the API, imparting necessary features for patient administration, and constructing desirable dried product attributes are the objective in developing an appropriate formulation. Coupled to those objectives are the concentration, dictating product presentation parameters of fill volume, as well as selection of the container and closure. The formulation and presentation both influence the conditions required for the lyophilization process.

In development, variations in critical process parameters are explored. Preparation for integrating a new product and the associated new procedures into manufacturing warrants a thorough understanding of the product and methods necessary to produce material. These manufacturing methods need to be effective and adequately controllable for both a small scale for early clinical studies, later to be refined for batches to be processed in routine manufacturing of commercial product. Manufacturing capabilities may be an existing in-house operation or at another facility providing contracted manufacturing services.

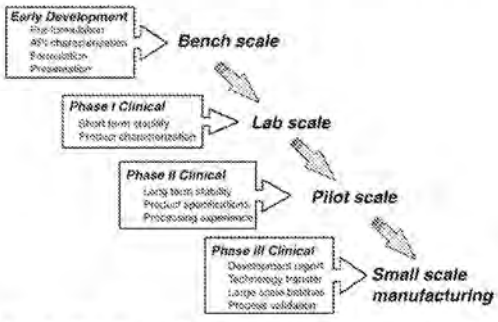


Fig. 1 Objectives and operations in progression to manufacturing scale.

Where the finished product is produced often influences the accessibility of information in learning the capabilities of manufacturing and the ease of technology transfer during the scale-up.

The focus in initial development of a potential new product is designing a product and suitable processing methods for the early clinical studies. With clinical success yet established, lyophilization process development is focused on short-term objectives and the efforts limited: time is of the essence. Results of early clinical studies direct refining the product design and processing procedures as clinical trials progress. Priority is given to designing a product presentation that is suitable for the intended route of administration, convenient to use with sufficient flexibility in dose administration in the clinic, and provides sufficient stability for the duration of the clinical studies. Often with limited knowledge, experience, and amounts of API at the time of early clinical studies, there is little opportunity for extensive product design and process development. The objective is to focus on an adequate product design for completing the studies and sufficiently conservative process for ease of technology transfer and scale-up.

At these early development stages, an "optimized" process is one that is safe, robust, efficient, and effective with minimal risk of difficulties in producing clinical materials, and not necessarily what would be considered the shortest. Challenges of transferring the product from early development to producing early clinical supplies involve availability of raw material, appropriate product design, and manufacturing capability. Often at the time clinical studies are beginning, processing methods for raw material are still evolving, the API material is not yet well characterized, and is in limited supply. With pressures to begin clinical studies, the timeline of a project is often accelerated and the transfer to a manufacturing facility is considered the last link in a series of efforts. With decisions made on the basis of only a few development studies and limited data, the objective of the scale-up of early clinical manufacturing is to manage the level of risk with a process that is suitable but not necessarily conducive for large-scale commercial manufacturing.

As clinical studies progress, development efforts can begin to focus on the final product design and process parameters established through expanded studies in the laboratory. Objectives for these studies become improvements in the procedures that are well suited for routine processing in a manufacturing environment. These activities would also include process validation studies. The scope and extent of the validation studies expand as batch sizes increase with the progression from development to commercial manufacturing. Fig. 2 provides a graphic representation of the development efforts and activities along with the extent of

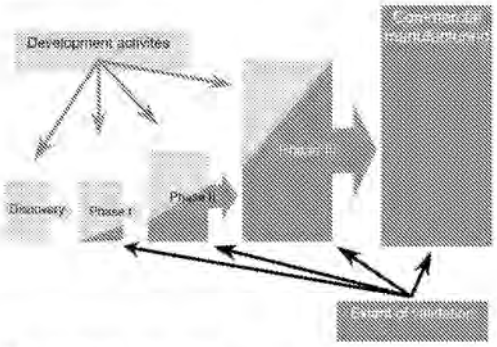


Fig. 2 Batch size, development efforts, and extent of validation.

validation appropriate as API production, finished product manufacturing, and analytical methods are refined in preparation for increasing batch sizes progressing toward large-scale commercial manufacturing.

The success of development efforts is measured by the ease of technology transfer and scale-up to large-scale manufacturing. Small-scale studies in the laboratory need to incorporate influencing factors of large-scale manufacturing. The intent is to develop a process that is safe, effective, efficient, and sufficiently robust where the manufacturing technology can be transferred to routine large-scale commercial production. Product-design objects are efficacy, stability, safety, and ease of use. Process design entails establishing desirable finished product attributes and ease of reproducible manufacturing that consistently results in high quality and yield, at a low unit cost. The formulation, product design, and process all have an influence on each other, with consideration of the impact of one on another being of significant importance during development.

PRODUCT DESIGN

Characteristics of the product and functions in early clinical studies are the driving force for initial product development. Often, there is a nominal knowledge of the API characteristics at such an early juncture. Preformulation studies provide attributes such as solubility, effects of pH, and predict sensitivities to environmental conditions. Solubility, along with pharmacologic characteristics, dictates the volume in the product container. Sensitivities to environmental conditions include temperature, light, oxygen, and contact surfaces. Specific to lyophilized preparations, there is also the potential for degradation via hydrolysis reactions when in the presence of water. Each of these aspects, in particular the sensitivities to environmental conditions, needs to be considered during preformulation and development studies in preparation for scale-up.

Aspects of selecting a product container include both initial fill volume for dispensing in manufacturing and volume used for reconstitution, along with ease of use in a clinical setting. Later considerations include batch size capacity in a lyophilizer during commercial manufacturing. Smaller-diameter containers yield increased number of units per shelf surface area in a lyophilizer, and therefore larger batch size. This has an impact on manufacturing unit costs as well as the ability to later meet growing market demands. There may be occasion that the container size changes as the product moves from early clinical studies to product approval for commercial manufacturing. Such changes should be expected and are often unavoidable. Source of the container may also change as the product is scaled up. It is therefore prudent to evaluate different sources of containers during development, qualifying at least a primary and a secondary supplier in preparation for routine manufacturing. This would also apply to elastomeric closures used to complete the primary barrier of the container-closure system for the finished product. Product compatibility, manufacturability, and container-closure integrity all need to be evaluated for both primary and alternate components, avoiding single-source materials.

FORMULATION DESIGN AND PRODUCT CHARACTERIZATION

The nature of the product, requirements for stabilization, route of administration, and processing are all factors that influence the design of the product formulation. Specific details of these aspects are beyond the scope of this presentation and have been addressed by Pikal elsewhere in this encyclopedia. As product development and scale-up occur through the progression of preparing material for clinical studies and subsequent commercial manufacturing, it is expected that a growing body of data is generated. Such data are important in characterizing the product and understanding the critical needs, sensitivities, and behavior of the product. Analysis of a lyophilized product provides insights that are helpful in scaling up to larger batch sizes with the potential of being processed in varying environments. Along with evaluating attributes of a lyophilized preparation, finished product specifications are developed.

As upstream manufacturing progresses from small- to larger-scale processing, quality attributes of the API material may change. These changes may influence the behavior in the finished product. Analysis of the API

for monitoring any changes encompasses potency and purity. Low-temperature thermal analysis is also warranted for assessing any impact of changes in the API. Changes in quality and purity can have an influence on the phase transition of the solution, influencing the behavior during freezing and the threshold temperature during drying. Quality and purity attributes of the raw material need to be evaluated for the impact on downstream processing and finished product attributes as the upstream processing for the API is scaled-up or significant changes are made.

The formulation and product presentation may require adjustments as the product progresses through clinical studies. Concentration of the active and dose content may be adjusted for materials in later clinical studies. If the changes are nominal, then there may be little need for adjustment to the process. Significant changes warrant further development as the lyophilization processing conditions require refinement and may require subsequent scale-up studies.

PRODUCT PREPARATION

Conditions having no apparent impact during development activities can become more significant during larger-scale manufacturing. These include length of time the product exists as an aqueous solution and exposure to widely different environmental conditions. As batch sizes increase through clinical materials preparation to large-scale manufacturing, the time interval from when the API is first compounded to form a solution to when the product is frozen and lyophilized becomes extended. A small batch may be formulated on the bench requiring only a few hours to compound, filter, fill, and begin lyophilization. In routine manufacturing, the bulk solution may be formulated and held for hours prior to sterile filtration and filling. The difference in time required for preparation of a batch may warrant reducing the temperature of the solution to minimize degradation reactions. If Arrhenius behavior applies for temperature dependence of rate constants for reactions, degradation would be significantly reduced by maintaining the bulk solution at 5°C during storage rather than 25°C. Such practices are appropriate when the stability of the product as a bulk solution is limited.

The vessel used for the compounding may be different during development simply because of batch size. Compatibility with these different materials is important to consider in preparation for scale-up. Bench-scale studies and small clinical batches may be compounded in glass Erlenmeyer flasks or glass carboys. Commercial-scale manufacturing would typically use larger stainless steel tanks. Compatibility with the different materials that the product comes into contact with is important to evaluate. Volume-to-surface-area ratios may change based on the configuration of the bulk solution container. This affects exposure to the air and absorption of oxygen and carbon dioxide. To limit the effect of such sensitivities as with oxygen, an antioxidant may be included in the formulation. Extent of exposure to light may also be different when preparing larger-quantity bulk solutions. Such sensitivities are important to explore in preformulation and early development studies and considered during scale-up.

LYOPHILIZATION PROCESS DEVELOPMENT

Part of the development studies focused on defining the critical process parameters to be controlled and that achieve reproducibility of the process and consistency of the finished product. An integral part of the development activities for a lyophilized product is establishing appropriate process parameters that encompass loading the liquid-filled containers through stoppering and unloading dried product from the lyophilizer. The lyophilization process encompasses loading, freezing, and any type of thermal treatment, primary and secondary drying, stoppering, and unloading. Critical processing parameters for these steps are shelf temperature, chamber pressure, and time. These parameters are considered to be independent as they are variables under direct control and affected only by the control implemented during the process. Table 1 outlines a process description that details the independent parameters used for processing a model presentation consisting of a lysozyme formulation representing a protein preparation. A corresponding recipe used in programming an automated control system is illustrated in Table 2. Fig. 3 represents a graphical recording from lyophilizing a batch when executing processing parameters outlined in Table 2. The graph illustrates the implementation of the independent parameters of shelf temperature, chamber pressure, and time, along with the resultant product temperature profile. Note that the product is maintained below a threshold temperature to assure drying with retention is accomplished. The sudden rise in product temperature, termed a "break," reflects the sublimation front passing the temperature sensor. If the sensor is located in the bottom of the container, then this break indicates that all of the ice in the container has sublimed. The product temperature then increases, approaches the shelf temperature, at which time it is appropriate to implement the parameters suitable for desorption in secondary drying. Understanding the impact of the critical process parameters on product behavior is important in developing a sufficiently robust process and controlling the variables during scale-up.

Numerous studies have demonstrated the impact of shelf temperature on product temperature and rates of

Table 1 Lyophilization process description: model presentation

Step	Processing parameters
Loading	Soak at 5°C (±5°) and 1 atm for 2 hr
Freezing	Ramp shelf to −40°C at an average controlled rate of 30°C/hr Control shelf at target set point of −40°C (±5°) for 3 hr
Primary drying	Evacuate chamber, control at a target set point of 80 μHg (±20 μHg). Ramp shelf to −10°C at an average controlled rate of 30°C/hr Control shelf at target set point of −10°C (±5°) for 15 hr
Secondary drying	Control chamber pressure at a target set point of 80 μHg (±20 μHg). Ramp shelf to 30°C at an average controlled rate of 30°C/hr Control shelf at target set point of 30°C (±5°) for 5 hr
Stoppering	Control chamber pressure at a target set point of 1 atm Control shelf at target set point of 30°C (±5°C).

sublimation. As DeLuca demonstrated in his studies presented in 1984, the effect is principally on the rate of sublimation, with some proportionate contribution to an increase in product temperature.[1] Summarized in Table 3, the data demonstrate the effect of increasing the shelf temperature by 10°C, yielding a substantial increase in sublimation, sometimes nearly doubling the rate. An increase in product temperature also resulted, although the product increased no more than 2 to 3°C. As sublimation consumes heat, the heat energy removed is replaced with that provided by the shelf. The higher the shelf temperature, the greater the amount of heat provided to the product, and therefore a greater potential rate of sublimation can be achieved. With heat from the shelf driving the rate of sublimation, the evolution of water vapor from the sublimation front and the product temperature resulting from attempting to reach steady-state conditions can sometimes be a fragile balance. A limit to the amount of heat supplied for promoting rates of sublimation is imparted by the dried layer above the sublimation front. Pikal et al. studied this relationship, modeling the effect of solutes in a formulation on the mass transfer of water vapor through the dried layer above the sublimation front.[2] In their studies Pikal et al. characterized models of crystalline and amorphous solutes predicting resistance to mass transfer of water vapor. They also noted influences of concentration to mass transfer. There has long been and continues to be interest in modeling the effects of these independent parameters of shelf temperature, chamber pressure, and time during loading and freezing through primary and secondary drying.[3–7] This interest is in both processing rates and impact on finished product attributes such as residual moisture.

Nail investigated the effect of chamber pressure on heat transfer and the relative rate of sublimation.[8] Chamber pressure has a prominent effect on product temperature. Shelf temperature is the principal influence on sublimation rate; chamber pressure has a strong influence on heat transfer from the shelf to the vial and the relative difference between the vapor pressure of ice and the environment. The combined effects yield a direct impact on product temperature. The driving force of sublimation is the difference between the vapor pressure of ice in the product and the pressure of the environment. An increase in the differential increases the propensity of ice to sublime. For example, if the product temperature is −32°C with a corresponding vapor pressure of ice being 321 μHg

Table 2 Lyophilization process control recipe: lysozyme model presentation

	Process conditions		
Step	Shelf temperature	Pressure	Segment time
Loading	Soak: 5°C	atm	2 hr
Freezing	Ramp: 30°/hr	atm	1.5 hr
	Soak: −40°C	atm	3 hr
Primary drying	Ramp: 30°C/hr	80 μHg	1 hr
	Soak: −10°CC	80 μHg	15 hr
Secondary drying	Ramp: 30°C/hr	80 μHg	0.6 hr
	Soak: 30°C	80 μHg	5 hr
Stoppering	Soak: 30°C	atm	Total: 27.2 hr

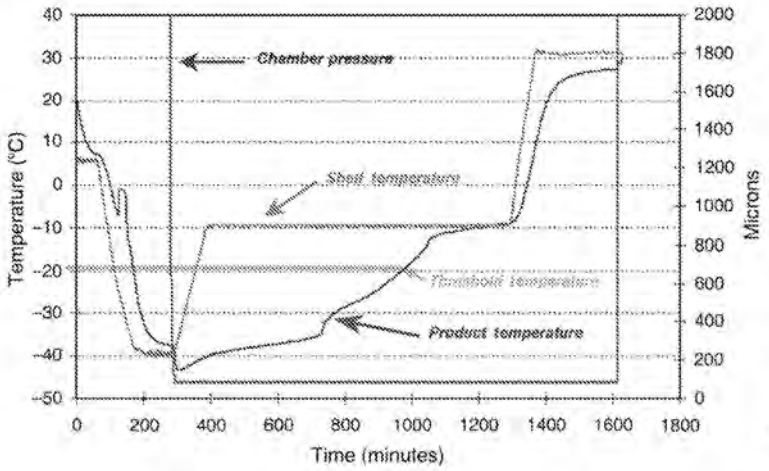


Fig. 3 Lyophilization process: lysozyme model preparation.

when the pressure in the product chamber is 300 μHg, the small pressure differential presents a weak driving force for the ice to vaporize. The increase in heat transfer along with the nominal rate of sublimation results in increased product temperature. With a chamber pressure of 50 μHg, a greater pressure differential would result in a stronger propensity for ice to vaporize. Greater sublimation coupled with reduced heat transfer due to lower pressure would therefore reduce the product temperature. Pikal et al. correlated the rate of sublimation to chamber pressure at a constant shelf temperature, as summarized in Table 4.[9] The magnitude of the difference between the chamber pressure and the vapor pressure of ice at the sublimation front and the pressure differential through the layer of dried solutes above the sublimation front have a significant impact on the mass flow of water vapor evolved from sublimation at the ice–vapor interface as it travels through the dried layer. As the ice–vapor interface forming the sublimation front progresses through the initial volume dispensed into the product container, the distance the water vapor travels increases. An increased pressure differential across this distance created with a lower chamber pressure improves the flow of water vapor through the dried layer. This, in turn, decreases the water vapor concentration at the ice–vapor interface, therefore increasing the propensity of ice to sublime.

All the factors that influence desorption to achieve sufficiently low residual moisture for complete drying are not yet well understood and are an area of increasing interest to investigators. Historically, conventional wisdom purports that a lower pressure readily removes any water bound to the product after the ice has sublimed. This water may be associated with solutes where the water may be part of the amorphous composition or simply adsorbed onto the surface of the solutes. Conditions of shelf temperature and chamber pressure and their effect on achieving low residual moisture are not yet well understood and of increasing interest. Studies by Pikal et al. suggest that reduced pressure in secondary drying does not have a significant effect on rates of desorption, at least for the model preparations and processing conditions studied.[10]

The time associated with the different conditions is also a critical independent processing parameter. Achieving complete solidification is assured with a sufficiently

Table 3 Predicting drying rate and product temperature at various shelf temperatures

Shelf temperature setpoint	Average product temperature	Average sublimation rate
50°C	−10°C	2.43 L/hr
40°C	−12°C	2.15 L/hr
30°C	−14°C	1.51 L/hr
20°C	−17°C	1.15 L/hr
10°C	−20°C	0.68 L/hr

(From Ref.[1].)

Table 4 Predicting drying rate and product temperature at various chamber pressures

Chamber pressure (μHg)	Average sublimation rate
400	0.55–0.61 g/hr
200	0.53–0.55 g/hr
100	0.42–0.45 g/hr
68	0.37–0.39 g/hr

Ranges for 20-mL tubing vials from different manufacturers. (From Ref.[9].)

low temperature for a minimum time. Factors that influence sublimation of ice during primary drying and achieving appropriate residual moisture content in secondary drying for adequate stability include sufficient time to complete the respective process step. The simplicity of time as a controlled variable is not to minimize the importance of the process parameter. Each of these three parameters, defined during development, is indeed critical and needs to be sufficient for processing, irrespective of what equipment and scale the product is processed. This includes processing at a smaller scale in early clinical materials processing or at a larger scale in routine manufacturing for marketed product.

Dependent process variables are those that result from implementing the various independent parameters under direct control during processing. As independent parameters are altered there will be some effect on the process conditions as reflected by the dependent variables. Proper control of independent variables will result in achieving the acceptable dependent variables, assuring a high level of confidence that the finished product exhibits the expected attributes. Adequately defined and executed process parameters would be expected to yield a finished product of predictable quality, purity, efficacy, and stability.

Product temperature is a principal dependent variable that reflects the progression throughout the lyophilization process. During freezing, the product is chilled to a final temperature for sufficient time to achieve the necessary solidification. The appropriate temperature required is determined by the character of the formulation and measured during thermal analysis studies completed as part of development. It is also prudent to verify the physicochemical properties if the product formulation is altered when the product progresses through clinical trials.

Even subtle differences in transferring and scaling up the process can have substantial effects in achieving this critical balance. Success during scale-up results from sufficient robustness instilled in the process during development. Initial process development activities for early clinical studies are indeed minimal. As clinical studies progress, further development work is appropriate where a process more suitable for routine manufacturing may be defined. This includes process robustness studies to establish viable ranges for critical parameters. Upon identifying target processing conditions for the independent variables of shelf temperature, chamber pressure, and time, further studies may focus on identifying suitable boundary conditions for establishing a proven acceptable range.[11] Variable processing conditions for such studies outlined in Table 5 and graphically represented in Fig. 4 reflect desirable ranges that yield a sufficiently robust process more easily integrated into a commercial manufacturing setting. These studies may be conducted as part of the development studies or treated separately as process validation activities. Considering these studies as either part of development or validation is not as important as the need to have completed such studies prior to scale-up to manufacturing. The greatest benefit is in having a process that is sufficiently robust where slight variations and influences due to scale-up and transfer become inconsequential.

FINISHED PRODUCT ATTRIBUTES

The attributes of the dried product are dictated by the nature of the formulation and conditions used during processing. It is therefore desirable, if not critical, that identical processing conditions be executed on a large scale such that the quality attributes achieved during development are replicated. Any differences in the processing conditions can result in differences in subsequent processing steps as well as finished product characteristics. A comprehensive product evaluation scheme would include analytical assessment of the

Table 5 Critical process parameter boundary conditions for a proven acceptable range

Process condition	Product loading	Cooling rate	Freezing	Primary ramp	Primary drying	Secondary ramp	Secondary drying
Shelf temperature:							
High	10°C	0.5°C/hr	−35°C	0.5°C/hr	−5°C	0.5°C/hr	35°C
Target	5°C	0.5°C/hr	−40°C	0.5°C/hr	−10°C	0.5°C/hr	30°C
Low	0°C	0.5°C/hr	−45°C	0.5°C/hr	−15°C	0.5°C/hr	25°C
Chamber pressure:							
High	atm	atm	atm	100 μHg	100 μHg	100 μHg	100 μHg
Target				80 μHg	80 μHg	80 μHg	80 μHg
Low				60 μHg	60 μHg	60 μHg	60 μHg
Time:	2 hr		3 hr		15 hr		5, 7, and 9 hr

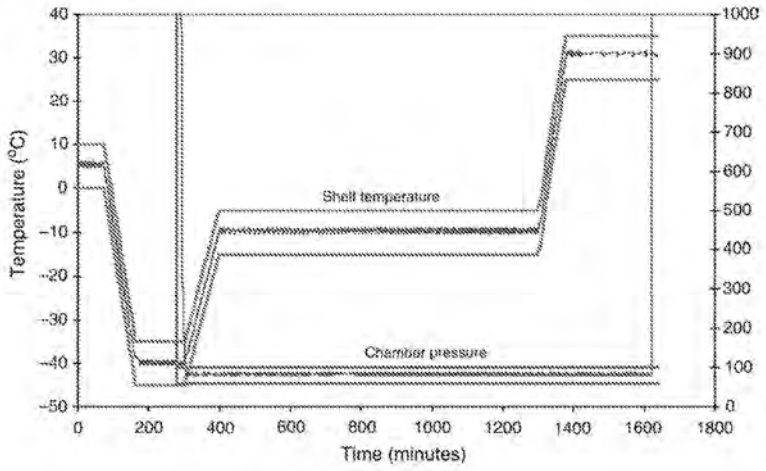


Fig. 4 Lyophilization proven acceptable range: lysozyme model preparation.

physicochemical product aspects along with those unique to lyophilized product. These include physical appearance, reconstitution time, and quality of the constituted solution, along with residual moisture.

Lyophilized preparations have a unique set of dried finished product attributes in addition to those of liquid, ready-to-use products.[12] Physical appearance of the dried cake, residual moisture, reconstitution time, and clarity of the constituted solution are product attributes important to quantify. The end result of lyophilization is to preserve attributes the product exhibited when first prepared after reconstitution of the dried product. Validation studies demonstrate and document the capability when starting materials are of a known acceptable quality and an appropriate and well-controlled process is reproduced, the finished product will be of predictable quality, purity, efficacy, and stability. As a minimum, chemical analysis may be used to show that the desired product attributes were preserved during processing and therefore the processing conditions were adequate. Measuring the potency of the product is important in verifying that both the filling and freeze drying operations were appropriate and controlled, producing a product with the required efficacy. It is also important to show that any degradation during processing has been avoided. Specific analytical techniques are used to assure this objective has been achieved and the product meets the predefined quality attributes.

The appearance of the dried product, in itself, is not considered a critical product attribute. It does, however, reflect success in processing and finished quality attributes. An objective and a measure of success of appropriate processing conditions is establishing and retaining the quality attributes imparted during freezing. Freeze drying with retention and avoidance of collapse through primary and secondary drying, as described by MacKenzie, reflects the level of success in proper processing.[13] If appropriate processing conditions are implemented that assure adequate solidification during freezing and the product is maintained below a threshold temperature during primary and secondary drying, the structure established during freezing will be preserved. Product warming above a threshold temperature where the material reverts to a liquid by either exceeding a glass transition causes a collapse of the structure or a eutectic temperature resulting in a melt. Either event alters the structure of the material and affects product behavior during further processing and finished product attributes. A change in the structure may impede sublimation and desorption, resulting in higher residual moisture, as well as influence reconstitution. Any change in the structure of the dried product is detected with physical inspection, assessing the dried cake appearance.

Physical appearance of the dried material can sometimes vary substantially. This is in part dependent upon the nature of the formulation along with techniques used in processing. The appearance of the dried cake is a mirror image of the ice crystal formed during the initial freezing. Fig. 5 illustrates the variation in dried product appearance due to differences in structure resulting from varying ice crystal size. If the ice crystals are large the cake may have a more coarse structure. Conversely, if the ice crystals are small, then the cake structure may be very fine. The structure of the ice is influenced by the shelf cooling rate during freezing. This, in turn, is dependent on the temperature at which the nucleation of ice occurs and on the rate of ice crystal growth. Searles et al. investigated such

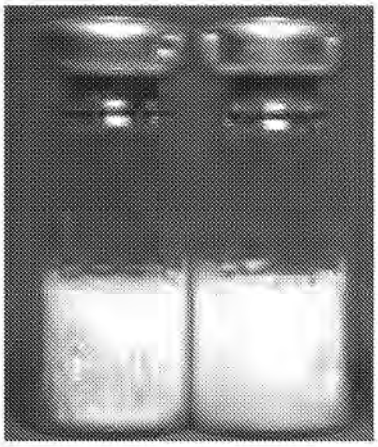


Fig. 5 Dried cake appearance.

influences of cooling rate and effect of thermal treatment on expected differences in ice crystal size.[14] If the cooling rate is different for the product processed in the manufacturing unit, then the physical appearance may be different.

The physical appearance is not considered a critical product attribute in itself. Rather, the physical appearance, reflecting attributes such as either a coarse or fine structure of the dried cake, may also be associated with other attributes such as reconstitution time (Fig. 5). For example, if large ice crystals form during the solidification as the product is chilled during freezing, large voids will remain in the dried product when the ice crystals have sublimed. These large voids then provide an avenue through which the diluent permeates the dried cake upon reconstitution. Changes in cooling rates with a potential effect on ice crystal size and the resultant change in dried product structure may therefore impart differences in ease of reconstitution. A time specification for the dried product to completely go into solution needs to be identified during development.

Ease of reconstitution is a desirable product attribute, although it is secondary to the quality, purity, efficacy, and stability. Reconstitution will sometimes indicate the presence of material that has collapsed and failed to dry with retention of the structure established during freezing. Collapse and melt back is associated with causing portions of the cake to dissolve more slowly and extend the time required to go into solution completely. The material polymerizing, peptides or proteins forming aggregates, or liposomes coupled or clustered may also be causes of a haze, cloudy solution or insoluble materials during reconstitution. Poorly soluble material or incomplete dissolution similar to that observed in Fig. 6 often indicates that there has been a significant physicochemical change in the product. The end point of the reconstitution is the formation of a clear solution, no less clear than the diluent used for reconstitution.

A target range of allowable residual moisture needs to be correlated to long-term storage stability and is necessary to define during development. Prevention of degradation by hydrolysis is circumvented by the removal of any appreciable water that may become involved in chemical reactions during storage. Stability in the dried state is assessed during development. The residual moisture of the dried product is dictated by the extent of desorption during secondary drying. The rate of desorption is strongly influenced by the product temperature and time in secondary drying. Achieving a residual moisture within the range established in development and correlated to processing conditions verifies that the time necessary for sufficient desorption was adequate when processing the larger batch size.

PROCESS DEVELOPMENT CONSIDERATIONS

There may often be many unknowns about suitable manufacturing operation while in the midst of product and process development activities. For preparation of clinical materials, the product may be produced in-house or at a contract facility. There are multiple possibilities for manufacturing product on a commercial scale: The product may be integrated into an existing in-house operation, manufactured at a yet unidentified

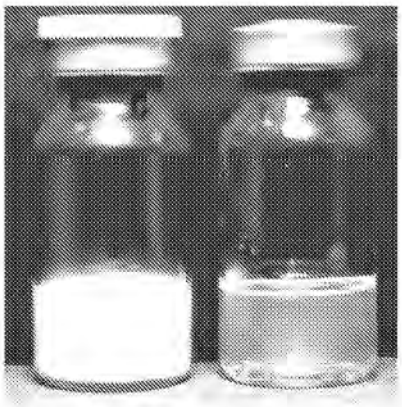


Fig. 6 Reconstituted solution.

contract manufacturing site, or the new product may warrant expansion of an existing operation or construction of a new facility. A direction may well be yet unknown at the time product and process development is ongoing. Such a circumstance requires some assumptions and foresight in selecting parameters when designing a process that would be best suited for routine large-scale manufacturing. Whether a manufacturing site is known or has yet to be identified, and unless there are extraordinary needs dictated by the product, designing a robust process increases the potential that technology transfer will be successful.

Intending that the process will be suitable for routine manufacturing, an understanding of an existing manufacturing operations capability provides valuable insight, direction, and focus to the development studies. With such understanding, the boundaries and parameters that envelope processing procedures suitable for the current manufacturing operation can be studied in development. If not existing, then an approximation of "typical" capabilities for a manufacturing operation is helpful. With planned expansion of operations required for commercial manufacturing, questions of "What do you need?" and "What can you provide?" for processing capabilities echo between development, engineering, and operations staff. To be complete and comprehensive, the perspective of this presentation on the topic will be development and scale-up into an existing operation. This will at least allow a comprehensive treatment for subjects of interest in scale-up. It is also important to realize that current operations should not necessarily be limited to critical product or processing needs. An example is with the use of organic solvents in a formulation to improve product structure or processing rates.[15,16] In such circumstances the need of stabilizing the product and impact on the process needs to be weighed in deciding on the direction during development. It is important to also appreciate that the current capabilities of any operation should not stifle creative and beneficial advancements in manufacturing technology.

For the first round of development it is prudent to attempt to fit a product and process within an existing operational capability. The first source of information to seek is the operational qualification (OQ) data quantifying the functional capabilities and performance of the lyophilizer. Table 6 outlines a list of performance capabilities of interest that allows a sufficient assessment of equipment functions and performance.

Shelf temperature has the greatest influence on the processing rates and ultimate product temperatures at the completion of each process step. The achievable shelf temperature capability ranges from the coldest temperature for freezing to the warmest temperature for primary and secondary drying. High rates of cooling and warming reflect the most aggressive changes in shelf temperature throughout the process. Control capabilities include the variation in controlling at a specific temperature and any relative deviation from the desired set point. This capability can also be influenced by the type and location of the temperature sensor. Shelf-temperature variation influences the batch uniformity and is important at the low temperatures used for freezing, intermediate temperatures often used for primary drying and relatively high temperatures for secondary drying.

Table 6 Performance capabilities from operational qualification studies

Shelf temperature:			
Range	Low		High
Rates	Cooling		Heating
Control	Low	Intermediate	High
Uniformity	Low	Intermediate	High
Chamber pressure:			
Range	Low		High
Control	Low	Intermediate	High
Sublimation/condensation rates:			
Shelf temperature setpoint			
Chamber pressure setpoint			
Rate achieved			
Total ice sublimed/condensed			

Left uncontrolled, chamber pressure reflects the water vapor in transit from the product from which ice is subliming to the condenser where the water vapor is converted back to ice. Chamber pressure has the greatest influence on product temperature. A pressure suitable for a formulation containing sucrose and having a phase transition or collapse temperature of $-32°C$ may be as low as $60\,\mu Hg$. Preparations with high phase transition temperatures such as those that contain mannitol that tends to crystallize and having a eutectic melt as warm as $-2.6°C$ may be processed using chamber pressures such as $400\,\mu Hg$. Controlling the camber pressure during primary drying has been widely used throughout the industry for more than 20 years, as noted previously. The effect and benefit of chamber pressure control during secondary drying is still a controversy.

There are three general methods used to control the chamber pressure. They include injecting nitrogen into the product chamber, injecting air or nitrogen into the inlet of the vacuum pump, and closing the valve between the chamber and condenser or condenser and vacuum pump to decrease the flow of water vapor. It is a common and preferred practice to control the chamber pressure by introducing filtered nitrogen into

the chamber using a proportional valve. The behavior of the nitrogen when injected into the chamber, relative effect on rates of sublimation during primary drying as compared to the other methods, influence on desorption during secondary drying, and effect on conversion of water vapor to ice at the condenser surface all need to be considered when utilizing pressure control by the various methods.

Sublimation and condensation rate studies are part of a comprehensive OQ and reflect the capability of the equipment to complete the primary drying. The amount of ice sublimed from the shelf and collected on the condenser with a constant shelf temperature and chamber pressure indicates the level of performance that can be achieved under load conditions. The rate reflects the capability of the shelves along with the heat transfer system to supply sufficient energy for sublimation. Evolution of corresponding quantities of water vapor challenges the effectiveness and capacity of the condensing system. The quantity of ice sublimed and condensed reflects both the obtainable rates and capacities. Sublimation of ice from the shelves reflects the capability to supply heat to the product. The amount of ice sublimed can also indicate the uniformity when rates from each shelf are compared. For an external condenser the pressure differential between the chamber and condenser reflects any inhibition to water vapor flux because of the equipment configuration when the vapor is in transit from the product to the condenser. The quantity of ice collected on the condenser is a measure of the effectiveness in trapping and removing water vapor and the ability to hold the expected quantity of ice. The sublimation and condensation rate studies are therefore useful in evaluating the equipment capabilities and gaining confidence that the lyophilizer can achieve the required process parameters.

Capabilities of the lyophilizer to be used for larger-scale manufacturing become the extent of processing parameters and therefore provide guidelines for process development studies. For transparent scale-up the parameters investigated during laboratory studies need to be within the known operating capabilities of the manufacturing equipment. Controlling the parameters investigated during process development to within the capabilities of the larger production unit eliminates the need for adjustments to the process when scaling up to larger batches in a manufacturing environment.

Validation data for large-scale processing in manufacturing is also a wealth of information on the processing capability and performance. Historical manufacturing experience with other products provides an indication of the extent of batch-to-batch variation during routine manufacturing. This would include an assessment of both processing capabilities as well as finished product characteristics. The level of control and reproducibility, along with consistency of finished product, can be evaluated to assess capabilities and predict results during scale-up. Batch uniformity with respect to variation that may be attributed to different positions in the lyophilizer included in validation studies can also be useful. Environmental influences and their effect on rates of sublimation relative to position on the shelf have been illustrated by Greiff.[17] In order to encompass the variation, a sufficiently large batch is necessary to evaluate the impact. As API is often in limited supply for development studies, a sufficiently large batch may consist of vials filled with only the excipients or use of a surrogate formulation. Realizing that the number of vials in the development study may be small, evaluating the uniformity in behavior during processing and finished product attributes provides insight on the expected variation throughout a larger-scale batch.

Uniformity in the product behavior during processing and consistency in finished product is strongly influenced by nucleation and ice crystal growth during freezing. The randomness of nucleation, an event based on probability, and subsequent ice crystal growth can be a significant influence when solidification is complete during freezing and product temperature during drying. Fig. 7 reflects the distribution of times and temperatures at which nucleation occurs for a limited number of vials within a small-scale batch being monitored using fine-gauge thermocouples carefully positioned in the center at the bottom. Note also the increase in the temperature of the monitored vials reflecting in a second and later increase in temperature due to nucleation and ice crystal growth in neighboring vials. This delay in the solidification of water in vials without a thermocouple suggests a greater extent in supercooling, imparting the associated attributes expected with smaller ice crystal size. This event occurs with a variation within any group of vials within any single batch. Such variation in nucleation within a group of containers is evidenced with a single batch and therefore within any group of batches. The range is apparent when comparing such events that occur among multiple batches using the same processing parameters. This variation is illustrated in Fig. 8 for four batches of a model formulation chilled using the same parameters.

This variation in product temperatures also occurs during primary drying as drying progresses and reflects what may be considered typical vial-to-vial variation during lyophilization. As ice crystal size dictates the surface area of the remaining solutes there may also be an effect on ease of removing residual moisture in secondary drying. For the same batch illustrated in Fig. 7 above and exhibiting a variation in nucleation, product temperatures will also vary during drying, as apparent in the temperature profile in Fig. 9.

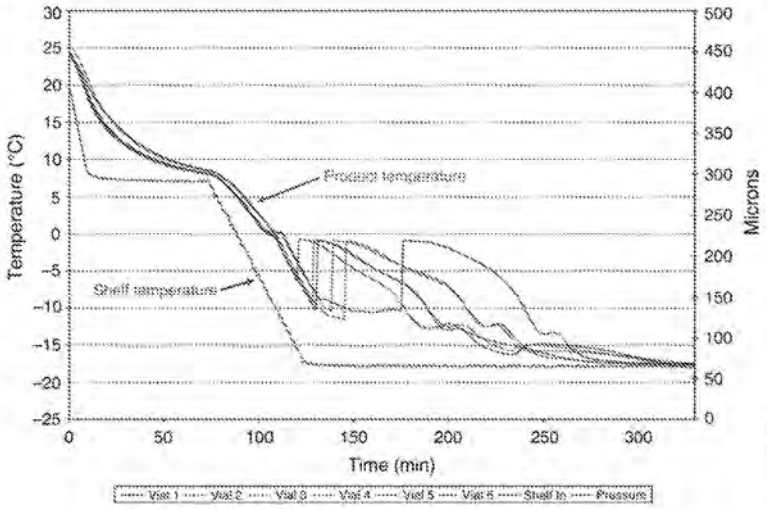


Fig. 7 Variation in time and temperature for nucleation and ice crystal growth within multiple vials of a single batch.

This vial-to-vial variation occurs, even in the most carefully controlled laboratory conditions. Such behavior will also occur in routine manufacturing and is important to consider while establishing parameters when developing a process. It is therefore imperative that the process be sufficiently robust to accommodate the inherent variation within a single batch and that occurs from batch to batch.

Compiling the data on equipment capabilities and experience of validation along with routine manufacturing provides a framework in which to select parameters for study during process development. Parameters controlled to within the operational capabilities of the manufacturing equipment minimized any adjustments to the process and continued development efforts during scale-up. Suitable parameters to accommodate variation that may occur within a larger lyophilizer and in routine manufacturing can be considered in the process design during development. Realizing that the product and process will exist in a manufacturing environment for the majority of the product life, the objective of the development studies

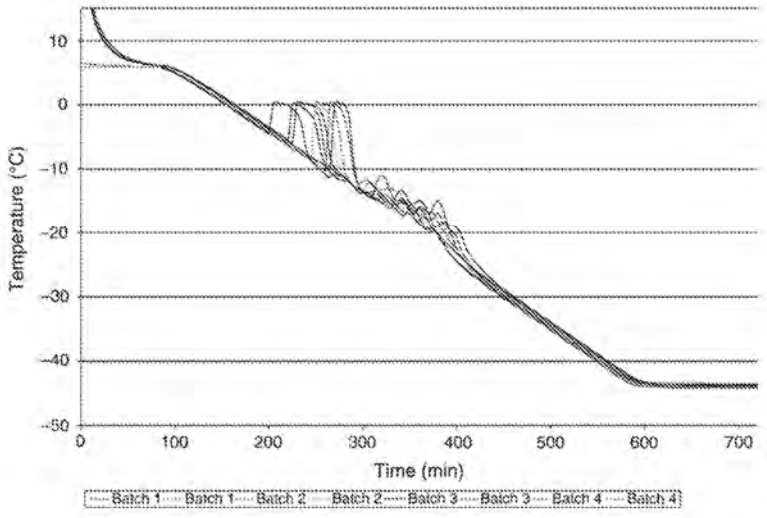


Fig. 8 Variation in time and temperature for nucleation and ice crystal growth within multiple vials of multiple batches.

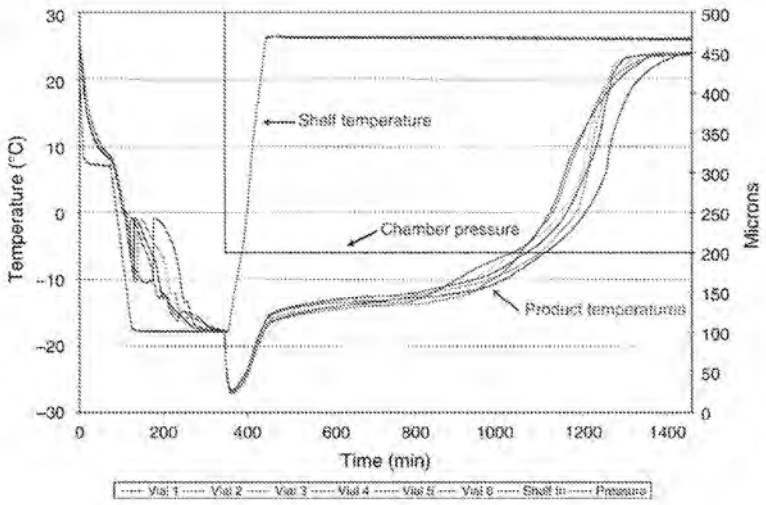


Fig. 9 Variation in behavior during processing multiple vials of a single batch.

is to establish a process that will easily integrate into a manufacturing operation during scale-up.

EQUIPMENT INFLUENCES

In circumstances where a new product is being integrated into existing manufacturing operations, the parameters of the process need to be within the manufacturing equipment performance capabilities. Therefore the capability of the equipment used during development and that to be used in manufacturing should be evaluated. This includes equipment design and configuration, control of critical processing parameters, and operating capacities. Understanding how the equipment for development and manufacturing functions and capabilities in implementing the required processing conditions is the first step in transferring a process from one operation to another.

It is not unusual that process development is complete before a manufacturing site is identified. Therefore the guidance for identifying appropriate process parameters for use in manufacturing may not be available. From a manufacturing perspective it is prudent to understand the processing capabilities for the research lyophilizer along with the actual parameters used, as well as any difference on the processing conditions the product experiences. For example, if left uncontrolled during development studies, cooling and heating rates can vary, dependent upon unit manufacturer, equipment component configuration, and lyophilizer capacity. When transferring a process, the processing conditions specified during development need to be replicated in routine manufacturing. Typical capabilities of the development unit that are important to assess are the same as those when evaluating large-scale manufacturing equipment, as presented in Table 5. Other considerations are instruments and methods used for process control and monitoring. A suitable production lyophilizer utilizes a resistance temperature device (RTD) located in a thermowell positioned in the heat transfer fluid just prior to where the fluid enters into the chamber and is the control point for the shelf temperature. This location is the most effective to use for control, as it provides the most consistent measurement, accommodating dynamics of the equipment, variations in processing, and differences in thermal load. Research units may have the sensor in a different location or a different method for monitoring and controlling the shelf and implementing temperature control. The impact of such a difference is the level of control as well as an offset to the actual shelf temperature. For example, an RTD located in a heat transfer fluid storage tank that is located upstream of the refrigeration heat exchanger and heater would reflect different dynamics and therefore a different "shelf" temperature.

A second major variable is method of pressure measurement. Research units often use thermoconductivity type pressure instruments to monitor the chamber pressure. These instruments were designed for measuring processes conducted at reduced pressures, such as those in the electronics industry. These instruments measure the differences in thermoconductivity of an atmosphere within the lyophilizer. Given the ideal gas law where $PV = nRT$, the pressure decreases

with a decrease in n, the number of molecules, where the molecular density, the number of molecules per unit volume is reduced, with all other variables constant. The thermoconductivity of an atmosphere decreases with a decrease in the molecular density and can be correlated to pressure. As these instruments are calibrated against nitrogen, the main constituent of a normal atmosphere, when sensing the pressure of a different atmosphere, such as that composed of water vapor, these types of instruments will indicate a different pressure. These instruments are prone to errors when the composition of the atmosphere varies. Sublimation during primary drying yields a composition of the atmosphere in the chamber high in water vapor, resulting in errors in measure pressure as high as 60%.

Position of the sensor can also have an influence on the indicated pressure. Monitoring and control of the pressure that comprises the environment to which the product is exposed is necessary. Therefore the sensor needs to be positioned on the product chamber. If the sensor is positioned on the condenser vessel or in the piping going to the vacuum pump, then the measured pressure will not reflect the amount contributed by the water vapor present during the sublimation of ice. Research lyophilizers sometimes have the pressure sensors located on the vacuum line and actually read the pressure of the pump rather than the chamber.

EXECUTING SCALE-UP ACTIVITIES

Knowledge of manufacturing capacities and capabilities, appropriate and adequate processing conditions for a robust process, coupled with careful control of starting material and processing conditions are necessary for successful technology transfer in achieving the predefined processing conditions and predicted finished product qualities. Sufficient knowledge of the product and process gained in development prevents scale-up from becoming a period of discovery. Processing at larger batch sizes provides an opportunity to verify the suitability of the processing parameters identified during development and focus on assessing the distribution of behavior and uniformity of finished product qualities at a larger scale.

A complete and comprehensive development report is an invaluable resource for technology transfer. This document preserves the body of knowledge and experience gained during product design and process development activities. Information including characteristics of the API, formulation, and finished product outlined in the preceding sections are part of a comprehensive development report. As well, the knowledge and experience in initial scale-up for processing clinical material is also valuable. The expected behavior, finished product attributes, and any difficulties that arise in scaling-up provide useful insight as larger batch sizes are integrated into routine manufacturing.

Realistically, subtle differences between conditions and outcomes during development and those in large-scale manufacturing will exist. Influences of preparing a product and processing in a manufacturing environment, in conjunction with the expanded quantity of finished product, combine to become significant factors. Batch sizes and quantities of finished units are often nominal as compared to that of large-scale manufacturing. Development studies and early clinical manufacturing requirements may only require relatively small batch sizes. As such, if the incidence of an event or result is one in a thousand and the batch size is a few hundred vials, it may only be an occasional observation or result. If the frequency is one in a few thousand, it may not become evident until large-scale batches are prepared in manufacturing. With manufacturing batch sizes approaching as large as tens of thousands of units being common, a statistically small incidence becomes a meaningful quantity of finished product.

The statistical distribution of variables simply due to larger quantities of finished product units will all be factors. These factors can be compounded such that any one alone has little or no impact. In combination and with potential of synergistic effects, the outcome of scale-up may yield results that are beyond any previous experiences. The level of success, even with factors or influences that may not be foreseen, is strongly dependent on the extent of knowledge and preparation during development as well as refinements implemented during the steps in preparation of clinical materials, leading up to integrating a new product into routine manufacturing for supplying the marketplace.

For scale-up of the lyophilization process, critical process parameters of shelf temperature, chamber pressure, and time are compared to those established during development and intended to be implemented using a larger lyophilizer. Some reasonable variation due to the dynamics of larger size and capacity equipment would be expected. For example, there may be an initial overshoot for a short duration when changing processing parameters. This is typical of proportional type control, where the amplitude and frequency of oscillations around the set point decreases until constant control at the set point is achieved. Proportional control is used for both shelf temperature and chamber pressure. These oscillations may be observed at the completion of a ramp leading to a controlled shelf temperature at a specific set point or when changing chamber pressure control, as in the transition from primary to secondary drying. These oscillations, if they are of nominal extent and duration, have little, if any, effect on product temperature during the process. Product during freezing and drying may also exhibit different

behavior. Placement of product temperature sensors is a major influence. Precise placement is relatively easily accomplished in a development laboratory setting. Using good aseptic technique in processing sterile product limits the manipulation necessary for specific placement of a sensor in a vial. Even with such difference in placement, accurate temperature values are assured at the most critical times in the process; the end of loading, freezing, primary, and secondary drying. It is important to verify the product has reached critical temperatures at the end of each of these steps to assure the conditions are adequate before progressing to the next step. Verifying that the product is chilled below the phase transition temperature and maintained below the threshold temperature identified during development are important assessments in evaluating the product temperature profile during scale-up batches.

Product behavior for a sterile product in manufacturing is also influenced by the conditions imparted due to requirements for aseptic processing. Materials prepared under laboratory conditions in a development environment are typically processed differently than in a manufacturing environment. For example, the level of cleanliness and bioburden are different. Product attributes, such as the nucleation and crystal growth of ice, would occur at different temperatures and rates. This has an influence on product behavior during processing and finished product attributes, as discussed earlier.

Verifying processing conditions are suitable and within reasonable range, finished product attributes are assessed and compared with expected results. Attributes associated with lyophilized preparations such as physical appearance, reconstitution, and residual moisture are important to evaluate and compare differences between results from development studies and those in a manufacturing environment. In addition, potency and purity, along with those attributes specific to a lyophilized dosage form, are compared to the specifications identified during development and refined as clinical studies progress.

Processing a larger number of units as batch sizes increase provides an opportunity to evaluate process capability. Beyond evaluating processing conditions and finished product attributes processed in a manufacturing environment, larger-scale batches provide an opportunity to evaluate the process dynamics and resulting product on a larger scale. It is prudent to evaluate a larger number of product samples during the initial large-scale manufacturing as compared to the relative limited number samples during routine manufacturing. With a larger number of samples, statistical methods provide a greater capability of quantifying the variation in product characteristics and quality attributes. Kieffer and Torbeck present methods and illustrations of establishing sample size and statistical approaches in conducting process capability studies using a step-by-step procedure useful for statistical evaluation when scaling up to larger batch sizes.[18] When processing larger batch sizes, reproducibility of processing conditions can be coupled with evaluating batch uniformity. This may include randomized sampling of a larger number of finished product units and using statistical methods to compare results within some desired distribution. Use of statistical methods provide a more quantitative measure at a larger scale for each scale-up batch and evaluate the results achieved between batches. This leads to accomplishing a higher degree of assurance as the product moves toward integrating the production into commercial manufacturing.

CONCLUSIONS

Ease and success of scale-up depends on the knowledge and experience gained beginning with development. Scaling up as the product progresses from development, through clinical studies, ultimately to large-scale commercial manufacturing occurs in multiple steps. During this progression there are often refinements to the product and process along the way. The starting materials should be well characterized. This includes the quality and purity of the API, adjustments in API, and formulation excipient concentrations, as well as the container-closure components. Requirements for preparing a batch and accommodating any product sensitivity need to be well defined for assuring the quality of the starting solution. A sufficiently robust process, with target parameters identifying "ideal" conditions, along with definition of a proven acceptable range within boundary conditions needs to be readily executed within a manufacturing environment.

The statistical distribution of variables due to larger quantities of finished product units can become a significant influence factor. This factor may be compounded such that any one alone has little or no impact. In combination and with potential of synergistic effects, the outcome of scale-up may yield results that are beyond any previous experiences. The level of success, even with factors or influences that may not be foreseen when preparing for scale-up in manufacturing as batch sizes increase, is strongly dependent on the extent of knowledge and preparation during development. This is also influenced by the extent of refinements implemented during the steps in preparation of clinical materials, leading up to integrating a new product into routine large-scale manufacturing.

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First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu			
Filer:	Jay Philip Lessler			
Attorney Docket Number:	104078.0001US			
Filed as Large Entity				
Filing Fees for				
Description	**Fee Code**	**Quantity**	**Amount**	**Sub-Total in USD($)**
Basic Filing:				
DOCUMENT FEE FOR THIRD-PARTY SUBMISSIONS	1818	1	180	180
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	180

Electronic Acknowledgement Receipt	
EFS ID:	43649474
Application Number:	17025881
International Application Number:	
Confirmation Number:	8113
Title of Invention:	Lyophilized Compositions of Phenobarbital Sodium Salt
First Named Inventor/Applicant Name:	Bala Tripura Sundari Chodavarapu
Customer Number:	149344
Filer:	Jay Philip Lessler
Filer Authorized By:	
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Payment was successfully received in RAM	$180
RAM confirmation Number	E20218UF46584281
Deposit Account	232185
Authorized User	Jay Lessler
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.16 (National application filing, search, and examination fees) 37 CFR 1.17 (Patent application and reexamination processing fees)	

37 CFR 1.19 (Document supply fees)

37 CFR 1.20 (Post Issuance fees)

37 CFR 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	1eng.pdf	2165184 dfda57c34b75386e38ad453293bcb740587 9068d	no	7
Warnings:					
Information:					
2	Non Patent Literature	1jp.pdf	2575150 ca539dfeba3b05f3da5a98abb4527e2b7569a9 380b3	no	7
Warnings:					
Information:					
3	Non Patent Literature	3.pdf	4841170 701d5d2bfbba865955 2aefa1e2a961427 6c9 74c5	no	10
Warnings:					
Information:					
4	Concise Description of Relevance	TPS.pdf	7359944 91347ff74e37f272f61e93a1bccb5bb955b72 aafc	no	29
Warnings:					
Information:					
5	Non Patent Literature	7.pdf	1495458 633a0b8fdd0828a1834cd583aa9b908625 835f1	no	5
Warnings:					
Information:					
6	Non Patent Literature	8.pdf	1018768 0a842e2103f8390914 2696cb1e47 3f6277 c625	no	3
Warnings:					
Information:					

7	Non Patent Literature	9.pdf	4555290 b4093df5fab0da3cb91d131b537307885bf6c892	no	8
Warnings:					
Information:					
8	Non Patent Literature	10.pdf	2975906 d2722f312a8b6d979f9456f96ab8973a283d3669	no	5
Warnings:					
Information:					
9	Non Patent Literature	4A.pdf	5150530 4c7f9e469a69680cb0b8e7d92acfff10982c9e33	no	6
Warnings:					
Information:					
10	Non Patent Literature	6A.pdf	10852525 6492fcf8bb89a5ff01b16f76eea0f41b2c7b49f9	no	127
Warnings:					
Information:					
11	Non Patent Literature	5A.pdf	4159014 32b7e31cd4002d3571192de8f89ab22e39e89a37	no	7
Warnings:					
Information:					
12	Non Patent Literature	2A-pt1.pdf	18495009 c79c26d94beede0a40d0a2f35293050 6eeb5f916	no	43
Warnings:					
Information:					
13	Non Patent Literature	2a-pt2.pdf	16550907 85d7dffb786e5a85e90abd02d0df85626886c6fb5	no	43
Warnings:					
Information:					

14	Concise Description of Relevance	Concise-description-generated.pdf	51764 13f5048337bc458a0ed7ed56567b392cfe463a03	no	11
Warnings:					
Information:					
15	Third-Party Submission Under 37 CFR 1.290	Third-party-preissuance-submission.pdf	74857 0077a6310ccd39c3e6ebba2285423406e785629b	no	5
Warnings:					
Information:					
16	Request for Notification of Non-compliant Third-Party Submission	Third-party-notification-request.pdf	23756 6383d287cb71a2df50da22926ac060e79c3b8bff	no	1
Warnings:					
Information:					
17	Fee Worksheet (SB06)	fee-info.pdf	37675 37e1528e499b99a81d51f8673d3e163224c94775	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	82382907		

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

B.T.S. Chodavarapu*et al.*

U.S. Patent Application No.: 17/025,881 Confirmation No.: 8113

Filed: September 18, 2020 Art Unit: 1617

For: LYOPHILIZED COMPOSITIONS OF PHENOBARBITAL SODIUM SALT Examiner: Y. Zhang

THIRD-PARTY SUBMISSION OF PRIOR ART UNDER 37 C.F.R. §1.290

Dear Sir/Madam:

A third party represented by the undersigned attorney (hereinafter "Third Party") hereby submits this Third Party Submission of Prior Art for consideration and entry in the above-identified application. This submission is timely because it is being filed prior to (1) a notice of allowance and (2) six months after the date on which the application was first published.

This submission includes (1) a document list identifying the documents relied upon in this submission, (2) a concise description of the relevance of each document listed, (3) legible copies of each document other than U.S. patents and U.S. patent application publications, and (4) English language translations of any non-English language documents identified in the document list.

Neither the Third Party nor the undersigned attorney is an individual who has a duty to disclose information with respect to the present application under 37 C.F.R. §1.56. This submission complies with the requirements of 35 U.S.C.§122(e) and 37 C.F.R. §1.290.

Submitted herewith is the fee of $180 required under 37 C.F.R. §1.17(o). No additional fee is believed due in connection with this submission. Please charge any shortage of fees due in connection with this Third Party Submission of Prior Art to Deposit Account No. 23-2185.

List of Documents Being Submitted as Third-Party Prior Art under 37 C.F.R. §1.290

1. K. Kawada, "Phenobarbital sodium lyophilized formulation: Overview of the clinical trial leading to approval of 'NOBELBAR®' and package insert information", Neonatal Care, Vol. 22, No. 7, pp. 657-663, 2009 ("Kawada-1")
2. Japan Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, "Deliberation Results Report" for Nobelbar for intravenous injection 250 mg, 43 pages, September 16, 2008 ("Nobelbar Deliberation Results Report")
3. Nobelpharma Co., Ltd., "NOBELBAR® 250 mg for Injection", version 12, November 2020 ("Nobelpharma")
4. K. Kawada et al., "A clinical Trial Assessing the Efficacy and Safety of a New Injectable Formula of Sodium Phenobarbital Containing No Additives for the Treatment of Neonatal Seizures", Jpn. J. Clin. Phamacol. Ther., Vol. 42, No. 6, pp. 205-10, July 2011 ("Kawada-2")
5. O. Bertaux et al., "Modification of hepatic alpha-1-glycoprotein and albumin gene expression in rats treated with phenobarbital", Eur. J. Biochem., Vol. 203, pp. 655-61, 1992 ("Bertaux")
6. J.H. Kern, "Freeze Drying as a Method of Processing Some Pharmaceutical Products", Doctor of Philosophy Thesis, The Ohio State University, 1954 ("Kern")
7. H. G. Brittain, "Polymorphisms in Pharmaceutical Solids", Marcel Dekker, Inc., New York, NY, 1999, ISBN 0-8247-0237-9 ("Brittain")

8. J. Bernstein, "Polymorphism in Molecular Crystals", Oxford University Press, Oxford, GB, 2002. ISBN 978-0-19-923656-5. ("Bernstein")

8. R.S. Fisher et al., "A practical clinical definition of epilepsy", Epilepsia, Vol. 55, No. 4, pp. 475-482, 2014. ("Fisher")

10. T. Fournier et al., "Phenobarbital Induction of α_1-Acid Glycoprotein in Primary Rat Hepatocyte Cultures", Hepatology, Vol. 20, No. 6, pp. 1584-88, 1994. ("Fournier")

11. "Access to Ohio State Dissertations and Theses", https://gradsch.osu.edu/access-distribution (accessed on July 14, 2021)

12. "OhioLINK: Frequently Asked Questions", https://etd.ohiolink.edu/apexprod/-f?p=1501:2:310637464866699 (accessed on July 14, 2021)

13. Entry from OhioLINK database for Kern, https://etd.ohiolink.edu/apexprod/-rws_olink/r/1501/10?clear=10&p10_accession_num=osu1486476025025146 (accessed on July 14, 2021)

14. U.S. Patent Publication No. 2017/0143719-A1 ("Parker")

15. Lyophilization of Parenteral (7/93) ("LoP")(https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-guides/lyophilization-parenteral-793) (dated November 11, 2014) (accessed on July 19, 2021)

16. U.S. Patent No. 8,436,190 ("the '190 Patent")

17. D.S. Przic, et al., "Lyophilization - The Process and Industrial Scale", Chem. Ind. Vol. 58, No. 12, pp. 552-562, 2004 ("Przic")

18. Encyclopedia of Pharmaceutical Technology (2007), Vol 1, Third Edition; James Swarbrick; Freeze; Freeze Drying, Scale-Up Considerations (Pg. 1807-1874) (" James")

19. I.B. Butler, et al. "Removal of dissolved oxygen from water: a comparison of four common techniques", Talanta, Vol. 41, No. 2, pp. 211-215, 1994 ("Butler")

20. R. Sandhyarani, "Development and Evaluation of Lyophilized Product of Apo-Acetozolamide", IOSR Journal of Pharmacy; Vol. 6, Issue 9 Version. 1, pp. 48-63, Sept. 2016 ("Sandhyarani")

Concise Description of the Relevance of Each Listed Document

1. **Kawada-1**

Kawada-1 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-1 at pp. 657 and 662. Kawada-1 teaches intravenous administration of NOBELBAR® solutions to neonatal (infant) patients for the treatment of neonatal convulsions and status epilepticus. *See id.* at p. 661. Kawada-1 discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. *See id.*

2. **Nobelbar Deliberation Results Report**

Nobelbar Deliberation Results Report is a 2008 review report conducted by Japan's Pharmaceutical and Medical Devices Agency (PDMA) regarding the drug NOBELBAR®, a lyophilized drug product for intravenous infusion containing 274 mg of phenobarbital sodium (equivalent to 250 mg phenobarbital free base) per vial, for which an application for approval was submitted by Nobel Pharma K.K. on September 14, 2007. Nobelbar Deliberation Results Report at p. 1. Nobelbar Deliberation Results Report found that NOBELBAR® had demonstrated efficacy and safety for treatment of neonatal convulsions and status epilepticus via intravenous administration. *See id.* at p. 2. The Nobelbar product was subject to long-term storage tests at 25°C and 60% relative humidity and under accelerated conditions (40° C and 75% relative humidity). *Id.* at 5. Based on the results of these storage tests, the expiration date of the Nobelbar product when stored at room temperature is 3 years. *Id.*

3. **Nobelpharma**

Nobelpharma discloses a phenobarbital sodium freeze dried preparation, NOBELBAR®. Nobelparma at p. 1. Nobelpharma discloses that NOBELBAR® was on sale as of December 2008. *Id.* Nobelpharma is version 12 of the document (revised November 2020), and shows revisions made in version 11 of the document (June 2019) as well. *Id.* NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* The powder is to be reconstituted prior to use by dissolution in water for injection or physiological saline to form a 50 mg/mL phenobarbital sodium solution. *See id.* at p. 4, right col (section 9(2)). Nobelpharma further discloses the phenobarbital sodium solution should be used promptly after dissolution. *See id.* Nobelpharma discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. Nobelpharma at p. 1, left col. and p. 5, left col. Nobelpharma teaches intravenous administration of NOBELBAR® solutions to neonatal (infant) patients for the treatment of seizures. *See* Nobelpharma at pp. 1 and 3-5.

4. **Kawada-2**

Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives, such as organic solvents (e.g., benzyl alcohol and propylene glycol). *See* Kawada at Abstract and p. 205 to p. 206 (left col., first paragraph). Kawada-2 teaches intravenous administration of NOBELBAR® solutions to neonatal (infant) patients for the treatment of seizures. *See id.* at Abstract and pp. 206-07. According to Kawada-2, NOBELBAR® has been marketed in Japan since December 2008. *Id.* at 210 (left col., first full paragraph).

5. Bertaux

Bertaux discloses a lyophilized sodium phenobarbital product (Gardenal for subcutaneous or intramuscular injection, Specia, Paris, France) and its reconstitution in apyrogenic sterile normal saline. Bertaux at p. 656, left col. Bertaux does not indicate the presence of any impurities in the sodium phenobarbital, whether in lyophilized or reconstituted form. Bertaux further teaches administration of the reconstituted lyophilized sodium phenobarbital to rats via subcutaneous injection. *See id.* Bertaux teaches administering 75 mg of phenobarbital per kg of the individual (here, a rat). *See id.*

6. Kern

Kern discloses the preparation of lyophilized phenobarbital. Kern at pp. 81-82. Kern further discloses that lyophilization (also known as freeze-drying) can greatly extend the life of labile products. Kern at p. 4 and 19.

The freeze-drying process involves freezing the product and a two-stage drying process. Kern at pp. 22-23. First, ice is evaporated from the frozen mass. Second, moisture is removed from the final dry solid leaving less than 0.5 percent moisture in the finished product. Kern at the paragraph spanning pages 22-23. Various products require different drying temperatures. Kern at 23. Vitamin preparations dry well at between -5 to -10° C and antibiotics at between -25 to -32° C.

Kern is a dissertation from Ohio State University dated 1954. Kern, cover page. According to Ohio State University Graduate School, dissertations for Ohio State are stored with OhioLINK.

See "Access to Ohio State Dissertations and Theses", https://gradsch.osu.edu/access-distribution (accessed on July 14, 2021). OhioLINK has an ETD Center, an online database of Ohio's undergraduate, masters and doctoral theses and dissertations from participating OhioLINK member schools. *See* "OhioLINK: Frequently Asked Questions", https://etd.ohiolink.edu/-apexprod/f?p=1501:2:310637464866699 (accessed on July 14, 2021). The ETD Center was launched in 2001. *Id.* Kern is available in the database and searchable at least based on its title and author. *See* the entry from OhioLINK database for Kern, https://etd.ohiolink.edu/apexprod/-rws_olink/r/1501/10?clear=10&p10_accession_num=osu1486476025025146 (accessed on July 14, 2021).

7. **Brittain**

Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. Brittain further teaches lyophilization can be used to convert crystalline materials to amorphous materials and rapid freezing is employed to form amorphous (noncrystalline) materials. *See id.* at pp. 214-215.

8. **Bernstein**

Bernstein teaches amorphous pharmaceutical materials are typically obtained from methods including lyophilization (freeze-drying). Bernstein at p. 254.

9. Fisher

Epilepsy is defined by two unprovoked seizures occurring at least 24 hours apart. *See* Fisher at Abstract and p. 476 (third paragraph).

10. Fournier

Fournier discloses a lyophilized sodium phenobarbital (referred to as Gardenal) for subcutaneous or intramuscular injection. *See* Fournier at p. 1584, right col. (penultimate paragraph). The lyophilized sodium phenobarbital was dissolved or diluted in a medium, specifically William's E medium. *See id.*

11. "Access to Ohio State Dissertations and Theses"

Dissertations for Ohio State are stored with OhioLINK.

12. "OhioLINK: Frequently Asked Questions"

OhioLINK has an ETD Center, an online database of Ohio's undergraduate, masters and doctoral theses and dissertations from participating OhioLINK member schools. The ETD Center was launched in 2001.

13. Entry from OhioLINK database for Kern

Kern is available in the database and searchable at least based on its title and author.

14. Parker

The presence of hydroxyl ions in aqueous phenobarbital sodium formulations results in a hydrolysis pathway. Parker at ¶4. The typical impurities for phenobarbital solutions that include water as part of the solvent system are phenyl ethyl acetyl urea (PEAU). *Id.* at ¶6. The impurity 2-ethyl-2-phenylmalonamide or alpha-phenylbutyrylguanidine forms as a degradation product from phenobarbital sodium. *Id.* at ¶7 and Table 1. The inclusion of significant amounts of water in a phenobarbital sodium formulation is not desirable as it will likely result in the formation of undesirable amounts of impurities. *Id.* at ¶16. Parker further teaches that phenobarbital sodium can degrade due to oxidation. *Id.* at ¶7.

15. Lyophilization of Parenteral (7/93) ("LoP")

Freeze-drying (or lyophilization) removes water form a product. LoP at 1. The lyophilization process includes dissolving the drug in a suitable solvent, generally water for injection. *Id.* at 1. Vials can be filled with a parenteral drug prior to lyophilization. *Id.* at 3. LoP also teaches that the freeze-drying process consists of freezing, primary drying (sublimination), and secondary drying (desorption). *Id.* at 1. More specifically, the process includes freezing a solution of water and a drug, applying a vacuum to the chamber (evacuating), and heating to evaporate the water from the frozen state (sublimation, primary drying). *Id.* at 1-2. Many lyophilization units include a secondary drying temperature and time. *Id.* at 6 (penultimate paragraph).

16. The '190 Patent

The '190 Patent discloses a lyophilization process for a product containing the drug bendamustine in which the product is subjected to drying at a pressure of about 10 to about 600 microns (Hg), which corresponds to about 10 to about 600 millitorr. '190 Patent at col. 17, line 63, to col. 18, line 14. The '190 Patent teaches initially drying at about -30 to -101° C and then at 25-40° C. *Id.* at col. 18, lines 14-27.

17. Przic

Przic presents a general overview of lyophilization and discusses the underlying principles of the process through the basics of: formulation, freezing, primary drying and secondary drying. Przic at Abstract. Przic teaches that the most extensive use of the lyophilization process is involved in the healthcare industry, specifically the lyophilization of pharmaceuticals such as parenterals. *Id.* at p. 557.

Przic defines lyophilization as a stabilizing process in which a substance is frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by desorption (secondary drying) to values that will no longer support biological growth or chemical reactions. *Id.* at Abstract. Przic teaches that an advantage of lyophilization is that the water content of the dried product can be reduced to very low levels. Generally, the lower the water content the more stable the product. *Id.* at p. 553, left col. Specifically, Przic teaches that water contents of 2% or less are achieved after the secondary drying step. *See id.* at p. 556, left col. Przic also teaches that the lyophilized product is normally sealed under vacuum or inert gas, greatly reducing oxidative denaturation. *Id.* at 553, left col.

Przic teaches that the formation of lyophilized pharmaceutical formulations starts by dispensing pharmaceutical solutions into glass vials or ampoules. *See id.* at p. 553, right col. After lyophilization is complete, the vials or ampoules are sealed to protect the lyophilized product from the environment. *See id.*; *see also id.* at p. 556, left col.

Finally, Przic discloses that phenobarbitone (also known as phenobarbital), marketed as "Fenobarbiton-Na" is lyophilized for use in healthcare. See Table 1 on p. 557, right col.

18. James

James provides a summary of freeze drying (or lyophilization) and its use as a drying process to convert solutions of labile materials into solids of sufficient stability for distribution and storage, as well as scale-up considerations for its use. James at p. 1807, left col., and p. 1834, left col. James teaches that, in a typical freeze drying process, aqueous drug solutions are first placed in glass vials and frozen at around -40°C to convert water into ice, and states that the drug is typically converted into an amorphous glass. *Id.* at p. 1807, left col. After freezing, the system is evacuated by vacuum pumps and a primary drying process is used to remove ice crystals via sublimation. *See id.* After the primary drying process, a secondary drying process is performed in the range of 25-50°C. *See id.* at p. 1807, right col. James teaches that freeze drying has historically been a method of choice for products intended for parenteral administration. *Id.* at 1808, left col. James also provides various model parameters for lyophilizing target compounds. *See id.* at p. 1838, Tables 1-2, and p. 1840, Table 5. Table 1 (p. 1838) teaches performing primary and secondary drying at 80 microns (Hg) (± 20 microns Hg), which corresponds 80 mTorr (± 20 mTorr).

19. Butler

Butler discloses techniques commonly used in the art for removing dissolved oxygen from water. Butler at Abstract. In one technique, Butler teaches the use of nitrogen gas purging, where nitrogen is bubbled into water 5-60 minutes at room temperature to provide water with an oxygen concentration ranging from about 0.19-0.56 ppm. *See id.* at p. 211, left col. to p. 212, right col., and Table 2; *see also id.* at p. 213, left col. ("Purging with nitrogen appears to be both a quick and effective procedure to scrub deionized water of dissolved oxygen.") and p. 214, left col. ("[T]he authors recommend use of pre-purified grade nitrogen, a sintered glass bubbler, high gas flow rates and an air-tight container (with a small vent hole to let the purge gas out) for effective removal of oxygen by this method."). Butler teaches that the removal of dissolved oxygen from water is important when experimenting with redox sensitive reagents. *Id.* at p. 211, left col.

20. Sandhyarani

Sandhyarani is directed to the development of a stable lyophilized formulation of the drug Apo-Acetazolamide. Sandhyarani at Abstract. Sandhyarani teaches lyophilization involves the removal of water or other solvent from a frozen product by a process called sublimation followed by desorption. *Id.*

As part of the manufacturing procedure, Sandhyarani teaches the formation of an Acetozolamide solution using Water for Injection having a temperature of 4-8°C prior to lyophilization. *Id.* at p. 51. The Acetozolamide solution, after a series of pH adjustment and

filtration steps, is filled into clear glass vials, subjected to lyophilization, and sealed in the vials under vacuum using stoppers and aluminum flip-off seals. *Id.* at pp. 51-52.

Below is a claim chart showing the relevance of the cited references.

Claim in U.S. Serial No. 17/025,881	**Reference(s)**
1. A pharmaceutical composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, wherein the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium is produced by: adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 and 9.6; and lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with water content at or below 1.1% wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0° C, followed by evacuating and secondary drying at a stable or declining temperature above 0° C.	Kawada-1 describes NOBELBAR® as a lyophilized pharmaceutical product of phenobarbital sodium. *See* Kawada-1 at pp. 657 and 662. Kawada-1 discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. Kawada-1 at p. 661. Nobelbar Deliberation Results Report is a review report conducted by Japan's Pharmaceutical and Medical Devices Agency (PDMA) regarding the drug NOBELBAR®, a lyophilized drug product for intravenous infusion containing 274 mg of phenobarbital sodium (250 mg as phenobarbital) per vial. Nobelbar Deliberation Results Report at p. 1. Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored under different conditions. *See id.* at p. 5. Nobelpharma discloses a phenobarbital sodium freeze dried preparation, NOBELBAR®. Nobelparma at p. 1. NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See id.* at p.4, right col. Nobelpharma discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2.

	Nobelpharma at p. 1, left col. and p. 5, left col. Kawada-2 discloses the use of NOBELBAR® for preparing an intravenous administration and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Bertaux discloses a lyophilized sodium phenobarbital product (Gardenal for subcutaneous or intramuscular injection, Specia, Paris, France) and its reconstitution in apyrogenic sterile normal saline. Bertaux at p. 656, left col. Bertaux does not indicate the presence of any impurities in the sodium phenobarbital, whether in lyophilized or reconstituted form. Kern discloses the preparation of lyophilized phenobarbital. Kern at pp. 81-82. Kern does not indicate the presence of any impurities in the phenobarbital, whether in lyophilized or reconstituted form. Kern also discloses that after the two-stage drying process, there is less than 0.5% moisture (water) in the finished product. Kern at the paragraph spanning pages 22-23. Various products require different drying temperatures. Kern at 23. Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. Brittain further teaches lyophilization can be used to convert crystalline materials to amorphous materials and rapid freezing is employed to form amorphous materials. *See id.* at 214-215.

	Bernstein teaches amorphous pharmaceutical materials are typically obtained from methods including lyophilization. Bernstein at. p. 254. The impurity phenyl ethyl acetyl urea (PEAU) forms from phenobarbital sodium in the presence of water. Parker at ¶6. Parker teaches that water causes hydrolysis of phenobarbital sodium, and the solution to this problem is removal of water from the formulation. *Id.* at ¶4 and 5. LoP teaches that freeze-drying (or lyophilization) removes water form a product. LoP at 1. LoP also teaches that the freeze-drying process consists of freezing, primary drying (sublimination), and secondary drying (desorption). LoP at 1. More specifically, the process includes freezing a solution of water and a drug, applying a vacuum to the chamber (evacuating), and heating to evaporate the water from the frozen state (sublimation, primary drying). LoP at 1-2. Many lyophilization units include a secondary drying temperature and time. LoP at 6 (penultimate paragraph). The '190 Patent teaches a lyophilization process in which initial drying is at about -30 to -101° C and then drying is at 25-40° C. '190 Patent at col. 18, lines 14-27. Przic defines lyophilization as a stabilizing process in which a substance is frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by desorption (secondary drying). Przic at Abstract. Przic teaches that an advantage of lyophilization is that the water content of the dried product can be reduced to very low levels; generally, the lower the water content the more stable the product. *Id.* at p. 553, left col. Specifically, Przic teaches that water

	contents of 2% or less are achieved after the secondary drying step. *See id.* at p. 556, left col. James teaches that, in a typical freeze drying process, aqueous drug solutions are first placed in glass vials and frozen at around -40°C to convert water into ice, and states that the drug is typically converted into an amorphous glass. James at p. 1807, left col. After freezing, the system is evacuated by vacuum pumps and a primary drying process is used to remove ice crystals via sublimation. *See id.* After the primary drying process, a secondary drying process is performed in the range of 25-50°C. *See id.* at p. 1807, right col. James teaches that freeze drying has historically been a method of choice for products intended for parenteral administration. *Id.* at 1808, left col. James also provides various model parameters for lyophilizing a target compound. *See id.* at p. 1838, Tables 1-2, and p. 1840, Table 5.
2. A method of producing a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium that forms no more than 0.2% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution, the method comprising: adding Phenobarbital Sodium to water to form a Phenobarbital Sodium solution with a pH at or between 9.3 to 9.6; and lyophilizing the Phenobarbital Sodium solution to produce the storage-stable powder form of lyophilized amorphous Phenobarbital Sodium with a water content at or below 1.1% wherein the lyophilizing comprises freezing, evacuating, and primary drying at a temperature below 0° C, followed by evacuating and secondary drying at a stable or declining temperature above 0° C.	Kawada-1 describes NOBELBAR® as a lyophilized pharmaceutical product of phenobarbital sodium. *See* Kawada-1 at pp. 657 and 662. Kawada-1 discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. Kawada-1 at p. 661. Nobelbar Deliberation Results Report is a review report conducted by Japan's Pharmaceutical and Medical Devices Agency (PDMA) regarding the drug NOBELBAR®, a lyophilized drug product for intravenous infusion containing 274 mg of phenobarbital sodium (250 mg as phenobarbital) per vial. Nobelbar Deliberation Results Report at p. 1. Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any

	changes when stored under different conditions. *See id.* at p. 5. Nobelpharma discloses a phenobarbital sodium freeze dried preparation, NOBELBAR®. Nobelparma at p. 1. NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See id.* at p.4, right col. Nobelpharma discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. Nobelpharma at p. 1, left col. and p. 5, left col. Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Bertaux discloses a lyophilized sodium phenobarbital product (Gardenal for subcutaneous or intramuscular injection, Specia, Paris, France) and its reconstitution in apyrogenic sterile normal saline. Bertaux at p. 656, left col. Bertaux does not indicate the presence of any impurities in the sodium phenobarbital, whether in lyophilized or reconstituted form. Kern discloses a method of producing lyophilized phenobarbital. Kern at pp. 81-82. Kern does not indicate the presence of any impurities in the phenobarbital, whether in lyophilized or reconstituted form. Kern also discloses that after the two-stage drying process, there is less than 0.5% moisture (water) in the finished product. Kern at the paragraph spanning pages 22-23. Various

	products require different drying temperatures. Kern at 23. Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. Brittain further teaches lyophilization can be used to convert crystalline materials to amorphous materials and rapid freezing is employed to form amorphous materials. *See id.* at 214-215. Bernstein teaches amorphous pharmaceutical materials are typically obtained from methods including lyophilization. Bernstein at. p. 254. LoP teaches that freeze-drying (or lyophilization) removes water form a product. LoP at 1. LoP also teaches that the freeze-drying process consists of freezing, primary drying (sublimination), and secondary drying (desorption). LoP at 1. More specifically, the process includes freezing a solution of water and a drug, applying a vacuum to the chamber (evacuating), and heating to evaporate the water from the frozen state (sublimation, primary drying). LoP at 1-2. Many lyophilization units include a secondary drying temperature and time. LoP at 6 (penultimate paragraph). The '190 Patent teaches a lyophilization process in which initial drying is at about -30 to -101° C and then drying is at 25-40° C. '190 Patent at col. 18, lines 14-27. Przic defines lyophilization as a stabilizing process in which a substance is frozen and then the quantity of the solvent is reduced first by sublimation (primary drying) and then by desorption (secondary drying). Przic at Abstract. Przic teaches that an advantage of

	lyophilization is that the water content of the dried product can be reduced to very low levels; generally, the lower the water content the more stable the product. *Id.* at p. 553, left col. Specifically, Przic teaches that water contents of 2% or less are achieved after the secondary drying step. *See id.* at p. 556, left col. James teaches that, in a typical freeze drying process, aqueous drug solutions are first placed in glass vials and frozen at around -40°C to convert water into ice, and states that the drug is typically converted into an amorphous glass. James at p. 1807, left col. After freezing, the systems is evacuated by vacuum pumps and a primary drying process is used to remove ice crystals via sublimation. *See id.* After the primary drying process, a secondary drying process is performed in the range of 25-50°C. *See id.* at p. 1807, right col. James teaches that freeze drying has historically been a method of choice for products intended for parenteral administration. *Id.* at 1808, left col. James also provides various model parameters for lyophilizing target compound. *See id.* at p. 1838, Tables 1-2, and p. 1840, Table 5.
4. The pharmaceutical composition of claim 1, wherein the water is Water for Injection sparged with nitrogen prior to adding the Phenobarbital Sodium.	LoP discloses using water for injection as the solvent in the lyophilization process. LoP at 1. Sandhyarani teaches the formation of an Acetozolamide solution using Water for Injection before lyophilization. Sandhyarani at p. 51. Parker teaches that phenobarbital sodium can degrade due to oxidation. Parker at ¶7. Butler discloses techniques commonly used in the art for removing dissolved oxygen from

	water. Butler at Abstract. In one technique, Butler teaches the use of nitrogen gas purging, where nitrogen is bubbled into water 5-60 minutes at room temperature to provide water with an oxygen concentration ranging from about 0.19-0.56 ppm. *See id.* at p. 211, left col. to p. 212, right col., and Table 2; *see also id.* at p. 213, left col. ("Purging with nitrogen appears to be both a quick and effective procedure to scrub deionized water of dissolved oxygen.") and p. 214, left col. ("[T]he authors recommend use of pre-purified grade nitrogen, a sintered glass bubbler, high gas flow rates and an air-tight container (with a small vent hole to let the purge gas out) for effective removal of oxygen by this method."). Butler teaches that the removal of dissolved oxygen from water is important when experimenting with redox sensitive reagents. *Id.* at p. 211, left col.
5. The pharmaceutical composition of claim 4, wherein the Water for Injection is sparged with nitrogen for at least 30 minutes prior to adding the Phenobarbital Sodium.	Same as claim 4.
6. The pharmaceutical composition of claim 1, wherein the water is cooled to or between 2 to 8°C prior to adding the Phenobarbital Sodium.	Sandhyarani teaches the formation of an Acetozolamide solution using Water for Injection having a temperature of 4-8°C before lyophilization. Sandhyarani at p. 51.
7. The pharmaceutical composition of claim 1, wherein the forming of the Phenobarbital Sodium solution with the pH at or between 9.2 to 10.2 comprises measuring the pH of the Phenobarbital Sodium solution, and wherein if the pH is higher than 10.2, the forming comprises adding Hydrochloric acid (HCl).	Kawada-1 and Nobelpharma discloses that an aqueous solution of phenobarbital sodium (1g phenobarbital sodium/10 mL water) has a pH of 9.2 to 10.2. Kawada-1 at p. 661; Nobelpharma at p. 1, left col. and p. 5, left col.
8. The pharmaceutical composition of claim 1, wherein the lyophilized Phenobarbital Sodium forms no more than 0.1 % phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after	Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored for 36 months at room temperature or 6 months under accelerated

storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).	stability conditions. *See* Nobelbar Deliberation Results Report at p. 5. Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See* Nobelpharma at p.4, right col. NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* at p. 1. Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Kern does not indicate the presence of any impurities in the phenobarbital, whether in lyophilized or reconstituted form. Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. The impurity phenyl ethyl acetyl urea (PEAU) forms from phenobarbital sodium in the presence of water. Parker at ¶6. Parker teaches that water causes hydrolysis of phenobarbital sodium, and the solution to this problem is removal of water from the formulation. *Id.* at ¶4 and 5.
9. The pharmaceutical composition of claim 1, wherein the lyophilized Phenobarbital Sodium forms no more than 0.05% phenylethylacetylurea (PEAU) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).	Same as claim 8.

10. The pharmaceutical composition of claim 1, wherein the lyophilized Phenobarbital Sodium forms no detectable amount of 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) when reconstituted in an aqueous solution after storage of the lyophilized Phenobarbital Sodium powder for up to 6 months at room temperature (RT) and accelerated stability conditions (ACC).	Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored for 36 months at room temperature or 6 months under accelerated stability conditions. *See* Nobelbar Deliberation Results Report at p. 5. Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See* Nobelpharma at p.4, right col. NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* at p. 1 Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Kern does not indicate the presence of any impurities in the phenobarbital, whether in lyophilized or reconstituted form. Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. The impurity 2-ethyl-2-phenylmalonamide (2EPMM) or alpha-phenylbutyrylguanidine (PBG) forms as a degradation product from phenobarbital sodium. Parker at ¶7 and Table 1. Parker teaches that water causes hydrolysis of phenobarbital sodium, and the solution to this problem is removal of water from the formulation. *Id.* at ¶4 and 5.
11. The pharmaceutical composition of claim 1, wherein the lyophilized Phenobarbital Sodium powder is stable up to at least 6 months at accelerated stability conditions	Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored for 36 months at room temperature or 6 months under accelerated

(ACC) which is equivalent to 24 months at room temperature (RT).	stability conditions. *See* Nobelbar Deliberation Results Report at p. 5. Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See* Nobelpharma at p.4, right col. NOBELBAR® is in the form of a powder that can be stored at room temperature. *See id.* at p. 1 Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Kern does not indicate the presence of any impurities in the phenobarbital, whether in lyophilized or reconstituted form. Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214.
12. The pharmaceutical composition of claim 11, wherein the stability of the lyophilized Phenobarbital Sodium powder is determined by adding the lyophilized Phenobarbital Sodium powder to an aqueous solution to form a reconstituted Phenobarbital Sodium solution and analyzing to quantitate the amount of phenylethylacetylurea (PEAU), 2-ethyl-2 phenylmalonamide (2EPMM), and/or alphaphenylbutyrylguanidine (PBG) in the reconstituted Phenobarbital Sodium solution.	Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored for 36 months at room temperature or 6 months under accelerated stability conditions. *See* Nobelbar Deliberation Results Report at p. 5. Nobelbar Deliberation Results Report discloses using, for example, identification tests (IR spectroscopy, and melting point) and purity tests (thin-layer chromatography and HPLC) for analysis. *See id.* Parker teaches analyzing the phenobarbital sodium solution to quantify the amount of impurities, such as PEAU. *See*, for example, ¶62-64 and Tables 3-6 of Parker.
13. The pharmaceutical composition of claim 1, wherein the Phenobarbital Sodium solution	Parker teaches that phenobarbital sodium is known to be soluble in water at

is at a concentration of 65 mg/mL, 100 mg/mL, 130 mg/mL or 200 mg/mL.	concentrations as high as 333 mg/mL to 1,000 mg/mL. Parker at ¶4.
14. The pharmaceutical composition of claim 1, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising filtering the Phenobarbital Sodium solution prior to lyophilizing.	LoP discloses dissolving a drug in a solvent and then filtering the solution through a 0.22 micron filter to sterilize it prior to lyophilization. LoP at 1 (second to last bulletpoint) and 2 (last paragraph).
15. The pharmaceutical composition of claim 1, wherein producing of lyophilized amorphous Phenobarbital Sodium powder further comprising: aliquoting the Phenobarbital Sodium solution into a vial prior to lyophilization; and after lyophilization, applying a closure to the vial under vacuum.	LoP discloses filling the vials prior to lyophilization. LoP at 3. Przic teaches that the formation of lyophilized pharmaceutical formulations starts by dispensing pharmaceutical solutions into glass vials or ampoules. *See* Przic at p. 553, right col. After lyophilization is complete, the vials or ampoules are sealed to protect the lyophilized product from the environment. *See id.*; *see also id.* at p. 556, left col.
16. A method of treating an individual in need of Phenobarbital Sodium, the method comprising: adding saline, water for injection, or a dextrose solution to a composition comprising a storage-stable powder form of lyophilized amorphous Phenobarbital Sodium as in claim 1 immediately prior to administration to form a Phenobarbital Sodium solution with no more than 0.2% phenylethylacetylurea (PEAU); and administering the Phenobarbital Sodium solution to the individual, wherein the efficacy of the treating is not diminished, and the side effects caused by degradation products of the Phenobarbital Sodium are not increased as a consequence of an extended storage duration of the Phenobarbital Sodium powder of at least 6 months at accelerated stability conditions	Kawada-1 describes NOBELBAR® as a lyophilized pharmaceutical product of phenobarbital sodium. *See* Kawada-1 at pp. 657 and 662. Kawada-1 teaches intravenous administration of NOBELBAR® solutions to neonatal (infant) patients for the treatment of neonatal convulsions and status epilepticus. *See id.* at p. 661. Nobelbar Deliberation Results Report discloses NOBELBAR® is a lyophilized phenobarbital sodium for the treatment of neonatal convulsions and status epilepticus via intravenous administration. Nobelbar Deliberation Results Report at pp. 2-3 and 19-20. Nobelbar Deliberation Results Report discloses NOBELBAR® does not exhibit any changes when stored for 36 months at room temperature or 6 months under accelerated stability conditions. *See* Nobelbar Deliberation Results Report at p. 5.

(ACC) which is equivalent to 24 months at room temperature (RT).	Nobelpharma discloses a phenobarbital sodium freeze dried preparation, NOBELBAR®. Nobelparma at p. 1. NOBELBAR® is in the form of a powder that is added to physiological saline to form a 50 mg/mL phenobarbital sodium solution for administration. *See id.* at p. 4, right col. Nobelpharma further discloses the phenobarbital sodium solution should be used promptly after dissolution of the freeze-dried preparation in the physiological saline. *See id.* Nobelpharma does not indicate any changes to the composition of NOBELBAR® during storage. *See id.* Kawada-2 discloses the use of NOBELBAR® and states it is a lyophilized product of phenobarbital sodium entirely free of additives. *See* Kawada-2 at Abstract and p. 205, left col. Kawada-1, Nobelpharma and Kawada-2 teach intravenous administration of NOBELBAR® solutions to neonatal (infant) patients. *See* Kawada-1 at p. 661, Nobelpharma at p. 1, left col. and Kawada-2 at Abstract and pp. 206-07. Bertaux discloses a lyophilized sodium phenobarbital (Gardenal for subcutaneous or intramuscular injection, Specia, Paris, France) and its reconstitution in apyrogenic sterile normal saline. Bertaux at p. 656, left col. Bertaux does not indicate the presence of any impurities in the sodium phenobarbital, whether in lyophilized or reconstituted form. Bertaux further teaches administration of the reconstituted lyophilized sodium phenobarbital to rats via subcutaneous injection. *See id.*

	Brittan teaches lyophilization is a useful technique for compounds that are susceptible to decomposition but are more stable as dry solids. *See* Brittain at pp. 213-214. Brittain further teaches lyophilization can be used to convert crystalline materials to amorphous materials and rapid freezing is employed to form amorphous materials. *See id.* at 214-215. Bernstein teaches amorphous pharmaceutical materials are typically obtained from methods including lyophilization. Bernstein at. p. 254. The impurity phenyl ethyl acetyl urea (PEAU) forms from phenobarbital sodium in the presence of water. Parker at ¶6. Parker teaches that water causes hydrolysis of phenobarbital sodium, and the solution to this problem is removal of water from the formulation. *Id.* at ¶4 and 5.
17. The method of claim 16, wherein the individual suffers from epilepsy.	Kawada-1, Nobelbar Deliberation Results Report, Nobelpharma and Kawada-2 teach intravenous administration of NOBELBAR® solutions to neonatal (infant) patients for the treatment of seizures. *See* Kawada-1 at p. 661, Nobelbar Deliberation Results Report at pp. 2-3, Nobelpharma at pp. 1 and 3-5 and Kawada-2 at Abstract and pp. 206-07. Epilepsy is defined by two unprovoked seizures occurring at least 24 hours apart. *See* Fisher at Abstract and 476 (third paragraph).
18. The method of claim 17, wherein the individual is a newborn suffering from neonatal epilepsy.	Same as claim 17.
19. The method of claim 16, wherein the composition is a powder dose of 65 mg, 100, 130 mg or 200 mg.	Kawada-1, Nobelbar Deliberation Results Report, Nobelpharma and Kawada-2 teach intravenous administration of NOBELBAR® to neonatal (infant) patients at a dose of 20 mg/kg. Kawada-1 at p. 661, Nobelbar

	Deliberation Results Report at pp. 2-3, Nobelpharma at p. 1, left col., and Kawada-2 at Abstract. Kawada-2 further discloses neonatal patients weighing between 2.121-3.796 kg, equating to dosages ranging from 42.42-75.92 mg phenobarbital sodium powder. Bertaux teaches administering 75 mg of phenobarbital per kg of the individual (here a rat) receiving said administration. Bertaux at p. 656, left col.
20. The method of claim 16, wherein the administering comprises intramuscular injection or intravenous injection.	Kawada-1, Nobelbar Deliberation Results Report, Nobelpharma and Kawada-2 teach intravenous administration of NOBELBAR® to neonatal (infant) patients. Kawada-1 at p. 661, Nobelbar Deliberation Results Report at pp. 2-3, Nobelpharma at p. 1, left col. and Kawada-2 at Abstract and pp. 206-07. Bertaux discloses a lyophilized sodium phenobarbital that can be administered via intramuscular injection. Bertaux at p. 656, left col.
24. The composition as in claim 1, wherein the steps of freezing and evacuation are conducted at a temperature of about -50° C and primary drying is conducted at a temperature increasing from about -50° C to 0° C.	During primary drying the temperature increases. '190 Patent, col. 18, lines 10-21. James teaches primary drying at around -40° C, and various model parameters for lyophilizing a target compound. *See* James at p. 1807, left col., p. 1838, Tables 1-2, and p. 1840, Table 5.
25. The composition as in claim 24, wherein the primary drying is conducted at least during a portion thereof at a temperature increasing from about -45° C to -35° C.	The '190 Patent teaches initially drying at about -30 to -101° C. '190 Patent at col. 18, lines 14-18. James provides primary drying at around -40° C, and various model parameters for lyophilizing a target compound. *See* James at p. 1807, left col., p. 1838, Tables 1-2, and p. 1840, Table 5.

26. The composition as in claim 1, wherein the secondary drying is conducted at least during a portion thereof at temperature between 25° C and 50° C.	The '190 Patent teaches initially drying at about -30 to -101° C and then at 25-40° C. '190 Patent at col. 18, lines 14-27. James teaches secondary drying processes are generally performed in the range of 25-50°C. James at p. 1807, right col. James provides various model parameters for lyophilizing target compounds. *See* James at p. 1838, Tables 1-2, and p. 1840, Table 5.
27. The composition as in claim 1, wherein the steps of evacuation, primary drying, and secondary drying are conducted at a vacuum of about 75 mTorr.	The '190 Patent teaches drying in a freeze-drying process at a pressure of about 10 to about 600 microns (Hg), which corresponds to about 10 to about 600 millitorr. '190 Patent at col. 17, line 63, to col. 18, line 14. James provides various model parameters for lyophilizing target compounds. *See* James at p. 1838, Tables 1-2, and p. 1840, Table 5. Table 1 (p. 1838) teaches performing primary and secondary drying at 80 microns (Hg) (± 20 microns Hg), which corresponds 80 mTorr (± 20 mTorr).

In view of the foregoing, it is respectfully requested that these documents be considered and made of record in the examination of the above-identified application.

Dated: August 31, 2021

Respectfully submitted,

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290
CONCISE DESCRIPTION OF RELEVANCE

Application Number	17025881

U.S. PATENTS

Cite No	Patent Number	Concise Description of Relevance

U.S. PATENT APPLICATION PUBLICATION

Cite No	Publication Number	Concise Description of Relevance

FOREIGN PATENT DOCUMENTS		
CiteNo	**Foreign Document Number**	**Concise Description of Relevance**

NON-PATENT PUBLICATIONS		
Cite No	**Reference**	**Concise Description of Relevance**
1	Nobelpharma Co., Ltd., NOBELBAR 250 mg for Injection, version 12, November 2020	See attached

2	J.H. Kern, Freeze Drying as a Method of Processing Some Pharmaceutical Products, Doctor of Philosophy Thesis, The Ohio State University, 1954	See attached

3	T. Fournier et al., Phenobarbital Induction of α1-Acid Glycoprotein in Primary Rat Hepatocyte Cultures, Hepatology, Vol. 20, No. 6, pp. 1584-88, 1994	See attached

4	H. G. Brittain, Polymorphisms in Pharmaceutical Solids, Marcel Dekker, Inc., New York, NY, 1999, ISBN 0-8247-0237-9	See Attached

5	Japan Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Deliberation Results Report for Nobelbar for intravenous injection 250 mg, 43 pages, September 16, 2008	See attached

6	J. Bernstein, Polymorphism in Molecular Crystals, Oxford University Press, Oxford, GB, 2002, ISBN 978-0-19-923656-5	See attached

7	O. Bertaux et al., Modification of hepatic alpha-1-glycoprotein and albumin gene expression in rats treated with phenobarbital, Eur. J. Biochem., Vol. 203, pp. 655-61, 1992	See attached

8	K. Kawada et al., A clinical Trial Assessing the Efficacy and Safety of a New Injectable Formula of Sodium Phenobarbital Containing No Additives for the Treatment of Neonatal Seizures, Jpn. J. Clin. Phamacol. Ther., Vol. 42, No. 6, pp. 205-10, July 2011	See attached

9	R.S. Fisher et al., A practical clinical definition of epilepsy, Epilepsia, Vol. 55, No. 4, pp. 475-482, 2014	See attached

10	K. Kawada, Phenobarbital sodium lyophilized formulation: Overview of the clinical trial leading to approval of NOBELBAR and package insert information, Neonatal Care, Vol. 22, No. 7, pp. 657-663, 2009	See attached

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

U.S. PATENTS				
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U.S. PATENT APPLICATION PUBLICATIONS				
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FOREIGN PATENTS AND PUBLISHED FOREIGN PATENT APPLICATIONS						
Cite No	Foreign Document Number[3]	Country Code[2]	Kind Code[1]	Publication Date (YYYY-MM-DD)	Applicant, Patentee or First Named Inventor	T[5]
						☐

NON-PATENT PUBLICATIONS (e.g., journal article, Office action)			
Cite No	Author (if any), title of the publication, page(s) being submitted, publication date, publisher (where available), place of publication (where available).	T[5]	E[6]

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

1	K. Kawada, Phenobarbital sodium lyophilized formulation: Overview of the clinical trial leading to approval of NOBELBAR and package insert information, Neonatal Care, Vol. 22, No. 7, pp. 657-663, 2009	☒	☐
2	R.S. Fisher et al., A practical clinical definition of epilepsy, Epilepsia, Vol. 55, No. 4, pp. 475-482, 2014	☐	☐
3	K. Kawada et al., A clinical Trial Assessing the Efficacy and Safety of a New Injectable Formula of Sodium Phenobarbital Containing No Additives for the Treatment of Neonatal Seizures, Jpn. J. Clin. Phamacol. Ther., Vol. 42, No. 6, pp. 205-10, July 2011	☐	☐

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4	O. Bertaux et al., Modification of hepatic alpha-1-glycoprotein and albumin gene expression in rats treated with phenobarbital, Eur. J. Biochem., Vol. 203, pp. 655-61, 1992	☐	☐
5	J. Bernstein, Polymorphism in Molecular Crystals, Oxford University Press, Oxford, GB, 2002, ISBN 978-0-19-923656-5	☐	☐
6	Japan Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Deliberation Results Report for Nobelbar for intravenous injection 250 mg, 43 pages, September 16, 2008	☒	☐

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THIRD-PARTY SUBMISSION UNDER 37 CFR 1.290	Application Number	17025881

7	H. G. Brittain, Polymorphisms in Pharmaceutical Solids, Marcel Dekker, Inc., New York, NY, 1999, ISBN 0-8247-0237-9	☐	☐
8	T. Fournier et al., Phenobarbital Induction of α1-Acid Glycoprotein in Primary Rat Hepatocyte Cultures, Hepatology, Vol. 20, No. 6, pp. 1584-88, 1994	☐	☐
9	J.H. Kern, Freeze Drying as a Method of Processing Some Pharmaceutical Products, Doctor of Philosophy Thesis, The Ohio State University, 1954	☐	☒

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10	Nobelpharma Co., Ltd., NOBELBAR 250 mg for Injection, version 12, November 2020	☒	☐

STATEMENTS

The party making the submission is not an individual who has a duty to disclose information with respect to the above-identified application under 37 CFR 1.56.

This submission complies with the requirements of 35 U.S.C. 122(e) and 37 CFR 1.290.

☒ The fee set forth in 37 CFR 1.290(f) has been submitted herewith.

☐ The fee set forth in 37 CFR 1.290(f) is not required because this submission lists three or fewer total items and, to the knowledge of the person signing the statement after making reasonable inquiry, this submission is the first and the only submission under 35 U.S.C 122(e) filed in the above-identified application by the party making the submission or by a party in privity with the party.

☐ This resubmission is being made responsive to a notification of non-compliance issued for an earlier filed third-party submission. The corrections in this resubmission are limited to addressing the non-compliance. As such, the party making this resubmission: (1) requests that the Office apply the previously-paid fee set forth in 37 CFR 1.290(f), or (2) states that no fee is required to accompany this resubmission as the undersigned is again making the fee exemption statement set forth in 37 CFR 1.290(g).

Signature	/Jay P Lessler/		
Name/Print	Jay P Lessler	Registration Number (if applicable)	41151

Examiner Signature		Date Considered	

*EXAMINER: Signature indicates all documents listed above have been considered,except for citations through which a line is drawn. Draw line through citation if not considered. Include a copy of this form with next communication to applicant. 1. If known, enter kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16. See MPEP 901.04(a). 2. Enter the country or patent office that issued the document, by two-letter code under WIPO standard ST.3. See MPEP 1851. 3. For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. 4. If known, enter the kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 . See MPEP 901.04(a). 5. Check mark indicates translation attached. 6. Check mark indicates evidence of publication attached.

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Document 1 (English translation)



Front Line of Neonatal Care

We would like to introduce this drug mainly to the parties involved in the release of NOBELBAR® 250mg for Injection (NOBELBAR® 250 mg for Injection, phenobarbital sodium lyophilized formulation) which was listed in the NHI price list as an anticonvulsant in December 2008.

Phenobarbital sodium lyophilized formulation

Overview of the clinical trial leading to approval of "NOBELBAR®" and package insert information

Kou Kawada*

Introduction

In 1992, the author started training at a university pediatrics department in order to be a physician in the Department of Neonatal Medicine. In April of the second year of training, the author who was assigned to the NICU was asked to inject phenobarbital® injection gently into severe asphyxia and very low birth weight infants, and to give an intramuscular injection to the outside of the thigh.

The patient is prospering from a pronounced instructor, and the number of patients is easily over 100. Several of the children who were examined by me passed away without treatment, and many children were well-growing. Some babies left lumps on the outside of their thighs that were too large to be called "dimples." It is doubtful whether the large quadriceps muscle injection, which is the big muscle of the very low birth weight infant, was the most appropriate method of administration and administration location for the phenobarbital preparation.

Phenobarbital has a property that is hard to dissolve in water. Phenobarbital® injection dissolves phenobarbital in organic solvents such as chlorobutanol to make it solution. The package insert of Phenobarbital® injection stated "intramuscular injection". A part of the drug solution that was injected intramuscularly leaked under the skin, and the tissue damage left a lump on the child's feet. Although the package insert states that "do not intravenously administer", it seems that the drug that was gently administered may have deposited in the blood vessels at the end of the route. This cannot be witnessed, and it is just an imagination. How do these crystals disappear?

The death of the child was due to the serious nature of the underlying disease, and the relationship between the death of the child and the drug administered intravenously was never mentioned. This was a picture of the NICU in Japan about 15 years ago.

The phenobarbital sodium lyophilized formulation NOBELBAR® 250mg for injection that is released in this article is easily dissolved in water, and the ingredient changes over time when dissolved. It is a relatively unstable phenobarbital sodium that is decomposed by about 6% in 24 hours after dissolution.

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The study journey

1. Off label use problem for children's medicines

The boss at that time was often told "breast milk" and "medicine" before he retired from college in order to properly build a relationship between parents and children, the authors stated that "how important is breastfeeding and mother-child interaction" and "how medicines for children are orphan drugs".

Even after retiring from university, the boss went to the Ministry of Health, Labor and Welfare (currently the Ministry of Health, Labor and Welfare) to solve the off-label use of drugs, and as a research project of the Ministry of Health, Labor and Welfare, he sought a way to escape from the "orphan" of children's medicine. After nearly a decade, this activity now highlights various off-label use problems. Among the many drugs, only a small portion of them have started to actually lead to the "off-label use" solution in the form of the description of the appropriate dose of acetoaminophen in the package insert, etc. Some of the results of this off-label use problem are also summarized on the website of the Japanese Society of Pediatric Clinical Pharmacology and Therapeutics[1].

The story goes back to Okayama in May 2004. This year, the author presented at the Japanese Society of Pediatrics academic conference held in Okayama on the "Effects of Prenatal Steroid Administration on CRIB II in Very Low Birth Weight Infants." A few years ago, it was reported a historical review of "theophylline formulation use was reduced by half with the introduction of Nasal CPAP in extremely low birth weight infants." This is the time when a large randomized comparative study was completed, mainly in Canada, which is "Caffeine reduces the risk of bronchopulmonary dysplasia and improves long-term prognosis"[2,3]. By the way, the results of this clinical study show that "the use of caffeine accelerates the use of Nasal CPAP by an average of one week"[2]. At that time, theophylline preparations Apnecur® and Apnission® were under public application in accordance with the PMSB/ELD Notification No. 104, "Handling of prescription drugs for off-label use", and caffeine preparations are still not subject to manufacturer development (approval) at that time. Without the activities of pharmaceutical companies, orphans would continue to be isolated.

2. For Investigator-Initiated Clinical Trials

There is a story about George Clooney[4], who is active in "Ocean's 11-13", going to Florida with a beautiful MR in Series I of his successful work "ER Emergency Room". The fictitious manufacturer (pharmaceutical company) that this MR is working for is "Nobel Pharma". Nobelpharma (in Japan), which was founded for the first year at that time, was a real venture company with ten or more people under the president. It is unknown whether the founding president knew the MR of "ER". This meeting in May 2004 was specifically decided to conduct an investigator-initiated clinical trial with Nobelpharma Co., Ltd., who was responsible for the development of NOBELBAR®.

In this meeting, people discussed what kind of clinical study (clinical study to be conducted on the assumption of application for approval) should be conducted. In addition, although investigator-initiated clinical trials were allowed under the system at that time, there was no precedent of drug approval, and there was no development fund other than the research project of the Japan Medical Association Center for Clinical Trials.

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At that time, the author did not expect that a doctor-initiated clinical trial would cost 210 million yen and a manufacturer development cost of 250 million yen, and that it would take a full four years to appraise the approval application. During that time, the author was unable to take care of the preparation and progress of the clinical trial, and the neonatal physicians were truly going to be assigned to the "on-site training for clinical trial work."

One month after this meeting, we have written the application for the research business application form of the Japan Medical Association Center for Clinical Trials for investigator-initiated clinical trials. I remember that the 32MB USB memory "Because the application form is included" was handed over and it was too late.

So what was the purpose of the anticonvulsant phenobarbital in newborns? When searching for the "Up to Date", which was introduced at the university at that time,

① Neonatal convulsions
② Sedation such as artificial ventilation
③ Brain protection in severely asphyxiated children, prevention of seizures
④ Enzyme induction for severe jaundice

and the like were listed. Looking back now, it is easy to understand that it is an extremely difficult to conduct the clinical trial other than "① Neonatal convulsion" in this list. "② Sedation such as artificial ventilation" is difficult to ensure the objectivity of the effect index, and "③ Brain protection and convulsions prevention for severely asphyxial children" causes frequent complications and deaths in severe cases, and the safety of medicine cannot be confirmed. In addition, it was difficult for the author at that time to see the effect of "④Enzyme induction for severe jaundice" as a single agent.

Looking at the clinical trial outline attached to the application form for the research project of the Japan Medical Association Center for Clinical Trials, there are not enough considerations and awareness regarding the safety of clinical trials (or drugs). The primary endpoint may have changed, or it may not be stated that a pharmacokinetic test will be conducted by measuring blood levels. On the other hand, the problems of using phenobarbital preparations in newborns at that time have been clearly pointed out. In other words, the development of safer formulations was the subject of this clinical trial, and was the background for the development of phenobarbital formulations in newborns at that time.

The following section clarifies the difference between phenobarbital injection and NOBELBAR® from the package insert.

Description in the package insert of phenobarbital preparation

1. Study Objectives

As for the purpose shown in the summary of planned clinical trials in the previous application, it is stated that "Phenobarbital is the most basic drug for anticonvulsants and sedatives in newborns and is routinely used in Japan and in the United States and Europe. In Japan, the drug product suitable for intravenous administration has not been marketed, and the drug product for intramuscular injection and subcutaneous injection have been used without any other means. The purpose of this clinical trial is to evaluate and confirm the efficacy and safety of the textbooks of phenobarbital IV for neonatal convulsions. The results obtained will be compared with reports in existing writing, literature, etc., and used as evaluation materials for application for approval of neonatal

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convulsion in Japan."

The study drug uses "generic name: Phenobarbital sodium", and the indication/effect to obtain approval is "neonatal convulsion." In the process of applying for approval of "NOBELBAR®," the indications for which approval is to be obtained include "Status epilepticus", and because development of a drug product that is suitable for use in children and adults is required, there is a history of using a relatively high dose of 250 mg drug product in newborns.

2. Problems with conventional phenobarbital preparations

The phenobarbital injections at that time were 10% phenobarbital® (approved by Fujinaga Pharmaceutical Co., Ltd.) and 10% phenobarbital injection "Marco" (same as Marco Pharmaceutical). Indications include "sedation of anxiety and tension (when urgently needed)", "autonomous seizures", "psychomotor seizures", and epileptic seizures (tonic seizures: generalized seizures : major seizures, focal seizures), including Jackson-type seizures[5], and the dosage and administration is "Phenobarbital sodium, usually 50 to 200 mg (1/2 to 2 mL once) for adults, is injected subcutaneously or intramuscularly once or twice a day. The dose may be adjusted according to the patient's age and symptoms." Other statements include pharmacokinetic data after intramuscular injection of 10.2 mg / kg (mean) of phenobarbital to 5 infants (5 months to 1 year and 6 months) in the package insert 10% Phenobarbital®. Not only that, both 10% Phenobarbital® and 10% phenobarbital injection "Marco" do not have any negative statements such as "safety has not been established" in the package insert. In the section of precautions for intramuscular injection, it is stated that "it is desirable not to use it continuously for infants, young children, and children." Clearly, the package insert of phenobarbital injection preparation at the time is stated on the assumption that the infant and children will also be administered.

However, the following problems were present. In Japan, phenobarbital preparations are not commercially available for intravenous administration, and intramuscular and subcutaneous preparations are being used for intravenous administration. It is administered even though the package insert states that "do not administer intravenously"[6]. The description in the package insert for this is as follows: "Because this drug precipitates the main drug with water, it should not be injected intravenously or mixed with other injected drugs" for 10% phenobarbital® and "intravenous injection" for 10% phenobarbital injection "Marco". Avoid mixing with other injections and use them separately." Although 10% Phenobarbital® for intramuscular injection contains organic solvents such as chlorobutanol and glycerin diethylether as additives in order to dissolve a poorly soluble main drug, administration of 10% phenobarbital for injection "Marco" containing benzyl alcohol, which is a similar organic solvent, is considered to be associated with the gasping syndrome due to benzyl alcohol toxicity, and when possible, it is preferable to use a drug product that does not contain these additives especially in newborns and low birth weight infants.

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