Journal of Pharmaceutical Sciences

AUGUST 1975

VOLUME 64 NUMBER 8

REVIEW ARTICLE

Characterization of Habits and Crystalline Modification of Solids and Their Pharmaceutical Applications

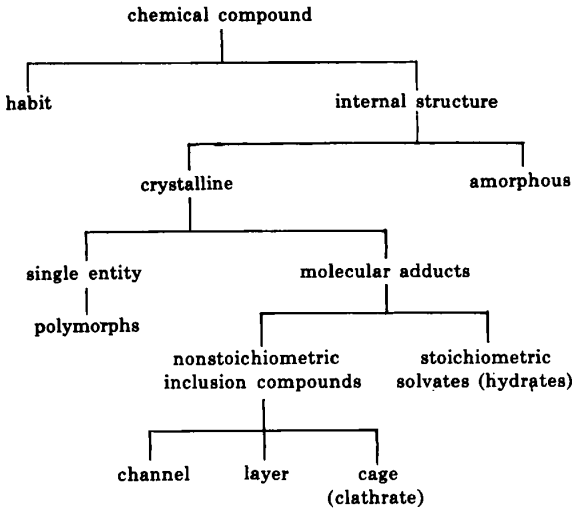
JOHN K. HALEBLIAN

Keyphrases □ Solids and their pharmaceutical applications—review of crystal habit, amorphous solids, polymorphs, solvates, and clathrates □ Crystalline aspects of pharmaceutical solids—review of crystal habit, amorphous solids, polymorphs, solvates, and clathrates □ Pharmaceutical applications of solids—review of crystal habit, amorphous solids, polymorphs, solvates, and clathrates □ Polymorphs—occurrence, dissolution kinetics, and pharmaceutical application, review □ Solvates—preparation, characterization, properties, and pharmaceutical applications, review □ Clathrates—crystal structure, preparation, characterization, properties, and pharmaceutical application, review □ Amorphous solids—preparation, characterization, and pharmaceutical application, review

CONTENTS

Physical Appearance of Solids-Habits 1270
Definition 1270
Factors that May Affect Crystal Habits 1270
Characterization of Habits 1271
Pharmaceutical Application of Habits 1272
Noncrystalline Solids-Amorphous Form 1272
Definition 1272
Preparation of Amorphous Solids 1272
Characterization of Amorphous Solids 1272
Pharmaceutical Application of Amorphous Solids 1273
Single-Entity Crystalline Solids-Polymorphs 1273
Examples of Polymorphism 1274
Dissolution Kinetics of Polymorphs 1275
Pharmaceutical Application of Polymorphism 1275
Stoichiometric Adducts-Solvates 1276
Definition 1276
Examples of Solvates 1276
Preparation of Solvates 1276
Characterization of Solvates 1278
Properties of Solvates 1279
Pharmaceutical Application of Solvates 1281
Nonstoichiometric Adducts-Clathrates 1283
Definition 1283
Crystal Structures and Examples of Clathrates 1283
Preparation of Clathrates 1284
Characterization of Clathrates 1284
Properties of Clathrates 1285
Pharmaceutical Application of Clathrates 1286
Conclusions 1286

Once the molecular chemistry of a potential drug is established and understood, most chemists feel that the compound is ready for formulation and clinical testing. Yet, every year, numerous papers appear showing that the performance of different drugs depends on, among other parameters, the habit and crystalline modifications of the active drugs. One crystal habit of a drug may tablet well while another may cause trouble, but both have the same melting point and apparently the same X-ray pattern. One crystalline modification may show five to 10 times the absolute solubility and bioavailability of another polymorph of the same drug. A drug marketed by Company A gives a stable suspension; the same com-



Scheme I—Outline Differentiating Habit and Crystal Chemistry of a Chemical Compound

pound, marketed by Company B, is stable only for a short time before the suspension is caked. These cases are not unusual. The majority of drugs marketed in various dosage forms probably can exist in different habits and crystalline modifications.

Since 1969, a series of review articles has dealt with polymorphism and its pharmaceutical application (1–5), but there is still confusion in the terminology used to identify different crystalline modifications of solids deviating from the basic definition of polymorphs (6–8). The intent of this work is not to review again the field of polymorphism but rather to review, in a broader sense, the different crystalline modifications of solids of pharmaceutical importance and their pharmaceutical application. Scheme I represents the outline to be followed in this review to differentiate habits and crystalline modifications of solids, *e.g.*, noncrystalline amorphous solids, polymorphs, solvates, and clathrates.

PHYSICAL APPEARANCE OF SOLIDS-HABITS

Definition—Habit is the description of the outer appearance of a crystal. If the environment of a growing crystal affects its external shape without changing its internal structure (which affects the polymorphism of a crystal), a different habit results. These

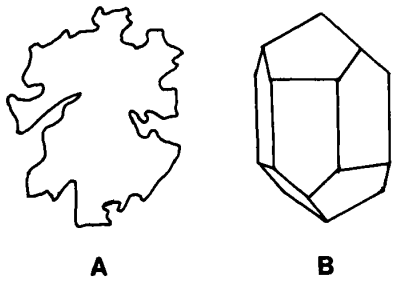


Figure 1—*Anhedral (A) and euhedral (B) quartz crystals. (Reproduced, with permission, from Ref. 9.)*

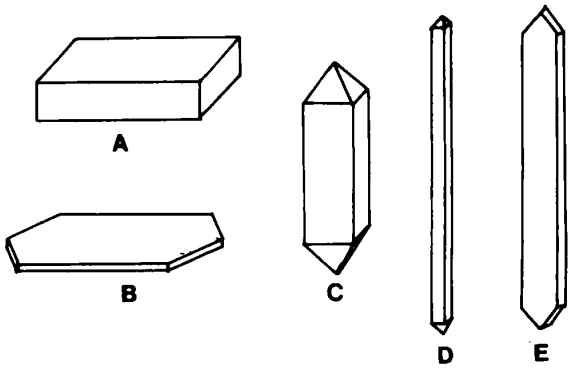


Figure 2—*Different habits of crystals. Key: A, tabular; B, platy; C, prismatic; D, acicular; and E, bladed. (Reproduced, with permission, from Ref. 9.)*

alterations are caused by the interference with the uniform approach of crystallizing molecules to the different faces of the crystal.

Crystal growth may be impeded by adjacent crystals growing simultaneously or contacting container walls. As a result, the development of plane faces may be inhibited or, in the case of late crystallizing crystals, an irregularly shaped crystal may occur since it is constrained to occupy only the spaces left between substances already crystallized. Such irregularly shaped crystals are described as anhedral or allotriomorphic (A, Fig. 1); those bound by plane faces are termed euhedral or idiomorphic (B, Fig. 1) (9). Anhedral crystals, although irregularly shaped, have a regular arrangement of building units which may be proved by X-ray diffraction.

Euhedral crystals are shown in Fig. 2:

A. Tabular crystal—moderate development of a pair of parallel faces, at the expense of the others, produces a tabular crystal.

B. Platy crystal—excessive development of the parallel faces as described in the tabular habit produces a platy crystal.

C. Prismatic crystal—crystal has a columnar form.

D. Acicular crystal—prism is elongated so much as to be needle like.

E. Bladed crystal—acicular crystal is flattened.

Factors that May Affect Crystal Habits—*Supersaturation*—The degree of supersaturation of the mother liquor or a supersaturation difference on opposite sides of the growing crystal may affect crystal habits. As supersaturation is increased, the crystal form tends to change from granular to needle like. A thin needle or dendrite loses less heat by conduction than a thicker crystal, so it grows faster. The effect of supersaturation on the change in the external form is given by a curve of high order which is characteristic of the substance:

$$y/x = k\Delta G^n \qquad \text{(Eq. 1)}$$

where y/x is the ratio of crystal length to breadth; k is a coefficient of proportionality which depends on the conditions of crystallization, especially on diffusion; G is the degree of supersaturation, in moles/

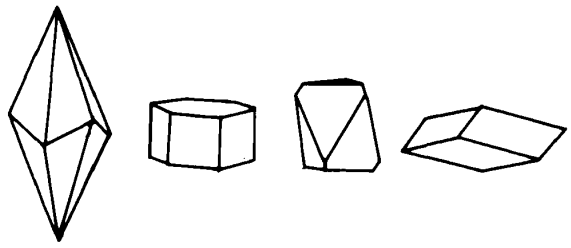


Figure 3—*Crystal habits of calcite. (Reproduced, with permission, from Ref. 9.)*

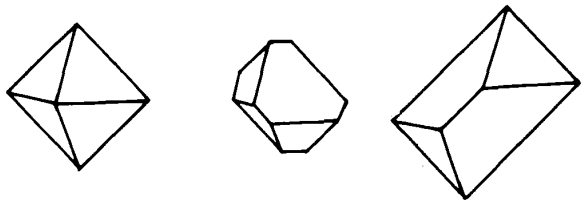


Figure 4—*Unevenly developed octahedra of magnetite. (Reproduced, with permission, from Ref. 9.)*

1000 moles of solvent, at the moment of formation of the nuclei of crystallization; and n is a number, above unity, dependent on the crystallographic classification and chemical composition of the substance (10).

Rate of Cooling and Degree of Solution Agitation—When naphthalene is rapidly crystallized by rapid cooling from ethanol or methanol, it gives thin plates; when it is slowly crystallized by evaporation, it yields compact crystals. The rate of cooling is effective in altering crystal habits because of its influence on the degree of supersaturation (11).

Nature of Crystallizing Solvent—The interaction between the solute and the solvent is important in controlling the crystal habit. For example, resorcinol crystallizes from benzene into fine needles, but it crystallizes from butyl acetate into squat prisms. Similarly, iodoform crystallizes as hexagonal bipyramids from aniline and as prisms from cyclohexane. This difference is due to the affinity of a solvated solvent to be adsorbed on certain crystal faces and thus to inhibit the growth of those particular crystalline faces (12).

Presence of Cosolutes, Cosolvents, and Adsorbable Foreign Ions—The addition of any new substance in a crystallizing medium may affect the habit of the crystal formed. Sodium chloride, which normally develops only cubic {100} faces, develops octahedral {111} faces when grown in the presence of urea (13, 14). Whetstone (15–18) studied crystal habit modifications due to the addition of impurities as crystal poisons. He found that the crystal habit change by dyes depended on anionic and cationic substituent groups and the nature of substitution. For further examples, the reader is referred to the review works of Tipson (11) and Biles (19).

Constancy of Conditions—Because any small change in these variables may affect the habit of the growing crystal, duplication of the habit of any crystal requires crystallization under identical conditions.

An excellent example of different habits of the same compound can be seen in calcite, $CaCO_3$ (20), where crystals can occur in acute or obtuse rhombs, hexagonal prisms, and many types of scalenohedra (Fig. 3). All of these geometric forms are built from the same unit rhombohedron of calcite, which can be shown readily by their optical properties and X-ray diffraction patterns.

Although habit is the description of the outer appearance of a crystal, crystals showing the same habit may appear different superficially. A good example is the three geometric forms of magnetite, Fe_3O_4, represented in Fig. 4, where all are octahedra of the cubic system but with uneven developments. Measurement of the interfacial angles would reveal true symmetry. Such measurements are the only morphological bases for crystal classification and identification of habits.

Characterization of Habits—The angle between two crystal faces can be described in two ways: (*a*) included or edge angle between two faces, and (*b*) interfacial or polar angle, the angle between the normals to the faces of the crystal (Fig. 5). The interfacial angle is commonly used in optical crystallography.

The interfacial angles of crystals are measured by instruments known as goniometers (9, 21). The simplest, the contact goniometer, is a semicircular protractor with a movable arm pivoted at the circle center. When the two faces enclosing the angle to be measured are fitted closely to the base and the movable arm, the value of the angle may be read off directly from the graduated half circle. Contact goniometers are used only on large crystals with dull faces for which the reflecting goniometer cannot be used.

In the reflecting goniometer (Fig. 6), the crystal to be measured is mounted on a stout needle. The needle is attached to the spindle of a graduated rotatable

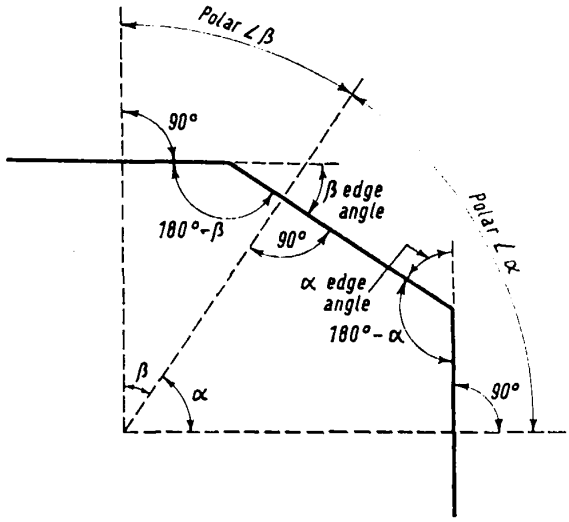


Figure 5—*Horizontal section across three vertical faces to show the relationship between included (edge) and polar angles. (Reproduced, with permission, from Ref. 9.)*

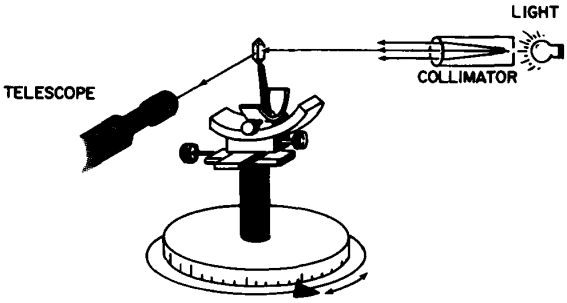


Figure 6—*Schematic of the reflecting goniometer.*

drum, which can be read to 1 min of arc by means of a vernier. The needle is provided with lateral and tilting adjustments, so that any zone of the crystal may be brought parallel to the axis of rotation of the drum. After this is done for one zone, parallel light from a collimator, fitted with a slit, is directed at the crystal and, by turning the drum, is reflected from each face of the zone in turn into a telescope provided with crosshairs. The reading is taken whenever the image of the slit coincides with the intersection of the crosshairs. The differences between these readings supply the angles between the normals to the faces (9).

Pharmaceutical Application of Habits—Crystal habits may influence several pharmaceutical characteristics.

Suspension Syringeability—The influence of suspension syringeability is mostly mechanical. For example, a suspension of plate-shaped crystals may be injected through a small needle with greater ease than one with needle-shaped crystals of the same overall dimensions (22).

Tableting Behavior—Shell (22), in his work on X-ray and crystallographic applications in pharmaceutical research, showed the effect of crystal habit on tablet properties. To evaluate the tableting behavior as influenced by crystal habit, he quantitatively described crystal habits by measurement of preferred orientation and related this parameter to compression characteristics of the powder. He took the ratio of the relative peak intensities of critical lines in the X-ray diffraction pattern and used them as average habits of crystals. In the compound studied, he found that the higher the 001 orientation ratio to the 010 orientation, the better was the tableting behavior.

Dissolution of Crystalline Material—If a crystal changes its habit due to crystal poisoning by a dye, then, in an indirect way, as reported by Piccolo and Tawashi (23), the adsorbed dye may inhibit the dissolution of drug crystals, which, in turn, may affect the bioavailability of the material.

Although the concept of habits of solids has been recognized since ancient times, the internal structure of solids, *i.e.*, the arrangements of atoms, was not understood until the introduction of X-ray crystallography by William and Lawrence Bragg. Solids in general are divided into two categories: (*a*) amorphous, where there is no regularity in the structure, and (*b*) crystalline, where the atoms are arranged in regular arrays. The crystalline form is the most frequent of the two and includes the metals and most minerals.

NONCRYSTALLINE SOLIDS-AMORPHOUS FORM

Definition—The most common amorphous solid is glass. Its atoms are put together in a nonuniform array compared to its crystalline form. Figure 7 represents the two forms of silica, which exists either as a crystal or as a glass. Cristobalite is the high-temperature form of quartz (SiO_2), while the glass structure looks like a distorted structure of cristobalite.

Randall and coworkers (24–28) reported that when a substance is capable of existing in both amorphous and crystalline forms, then the X-ray pattern given by the amorphous form may be regarded as a very diffuse version of the crystal pattern. There is, in fact, no sharp distinction between crystalline and amorphous states. If, starting with a coarsely crystalline solid, the size of the crystals could be reduced by stages and an X-ray diffraction photograph could be taken at each stage, the photographs would become diffuse when the crystal size fell below about 10^{-5} cm. With reduction of crystal size, the reflections become increasingly diffuse until the limit is reached at 10^{-7}–10^{-8} cm, the region of atomic dimensions, where the word crystal, with its implication of precise pattern repetition, ceases to be appropriate.

One cannot speak of a crystal only one unit cell in diameter, because the term unit cell implies repetition; this is the justification for the use of the term amorphous in describing glass-like substances, although the word noncrystalline might be preferable.

Preparation of Amorphous Solids—Preparation of amorphous solids, if feasible, does not follow a routine procedure. Methods mentioned by previous workers include lyophilization of fluprednisolone in *tert*-butanol (29), rapid quenching of chloramphenicol palmitate solution in hydrophilic solvents (30), and rapid quenching of melted chloramphenicol palmitate in the refrigerator to −10° (31). Precipitation is also used to prepare the amorphous prompt insulin zinc suspension of USP XVIII (32). It is prepared by precipitation of insulin and zinc chloride at a specified pH.

Characterization of Amorphous Solids—The only positive way to differentiate amorphous from crystalline solids is by means of X-ray powder diffraction. The X-ray powder diffraction of amorphous

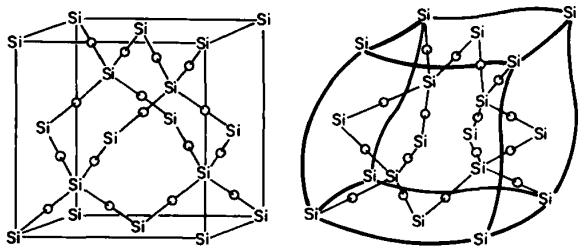


Figure 7—*Two forms of silica: crystobalite crystalline (left) and glass amorphous (right).*

Table I—Novobiocin Plasma Levels in Dogs following Oral Administration of Different Solid Forms[a]

Hours after Dose	Novobiocin Sodium, μg/ml Plasma	Amorphous Novobiocin Acid, μg/ml Plasma	Crystalline Novobiocin Acid
0.5	0.5	5.0	N.D.[b]
1	0.5	40.6	N.D.[b]
2	14.6	29.3	N.D.[b]
3	22.2	22.3	N.D.[b]
4	16.9	23.7	N.D.[b]
5	10.4	20.2	N.D.[b]
6	6.4	17.5	N.D.[b]

[a] Dose = 12.5 mg/kg. [b] Not detectable.

solids gives very diffuse reflections where the *d* distances, the distance between parallel planes in which the atoms of the crystal lie, cannot be determined as is done with crystalline solids.

Pharmaceutical Application of Amorphous Solids—Although it is feasible to prepare amorphous solids, there are few instances where advantage has been taken of this class of compounds. One main reason is their thermodynamic instability, since they are the most energetic form and tend to revert to a more stable form. This is particularly true when the formulation is in aqueous suspension.

Mullins and Macek (33), working on pharmaceutical properties of novobiocin, identified two forms of novobiocin: crystalline and amorphous. In tablet and capsule formulations, novobiocin is used as the sodium salt, which is active orally but is unstable chemically in a solution. The insoluble forms of novobiocin acid are more stable chemically. But the crystalline novobiocin acid is poorly absorbed and does not provide therapeutically adequate systemic levels following oral administration. The amorphous acid is readily absorbed and is therapeutically active. This difference in availability is due to differences in solubility in aqueous systems.

When an excess of crystalline or amorphous novobiocin acid in less than 10-μm size was shaken in 0.1 *N* hydrochloric acid at 25°, the amorphous solids were at least 10 times more soluble than the crystalline acid. This difference in solubility might be expected to favor the absorption of the amorphous solid from the GI tract. Data showing differences in novobiocin plasma levels following oral administration of 12.5 mg/kg each of amorphous novobiocin acid, crystalline novobiocin acid, and the sodium salt are shown in Table I.

Unless special precautions are taken to maintain the solid in suspension in the amorphous state by the addition of materials to suppress crystallization, amorphous novobiocin converts slowly to a crystalline form. The formulation becomes less and less absorbable and finally loses therapeutic effect entirely. A search was made for additives that would significantly retard or even prevent crystallization of aqueous suspensions of amorphous novobiocin, and some agents provided adequate protection for significant periods. The best agents found were methylcellulose, polyvinylpyrrolidone, and several alginic acid derivatives such as sodium alginate and propylene glycol algin.

In formulating injectable insulin, the duration of the action is controlled by its crystallinity. Insulin precipitates as an insoluble complex when reacted with zinc chloride and, depending on the pH, it may precipitate either as an amorphous or crystalline phase. Prompt insulin zinc suspension USP consists of amorphous insulin zinc complex. It is readily absorbed when injected and has a relatively short duration of action.

Extended insulin zinc suspension USP is made up of crystalline zinc complex. It is very slowly absorbed and has a longer duration of action. Insulin zinc suspension USP is made up of a mixture containing seven parts of crystalline and three parts of amorphous insulin zinc complex, and it is intermediate in duration of action. Another difference in the formulations is their particle sizes; prompt insulin is made up of small particles, and extended insulin is made up of large particles. This is another example where the rate of absorption and duration of action are determined both by particle size and degree of crystallinity (32).

Crystalline solids produced by the different methods of crystallization may crystallize out as single entities or as molecular adducts, where the adduct is one of the solvents of crystallization. The solid crystalline phase of a single entity is said to be polymorphic when it is crystallized at least in two different arrangements of the molecules.

SINGLE-ENTITY CRYSTALLINE SOLIDS-POLYMORPHS

Haleblian and McCrone (1) reviewed the pharmaceutical applications of polymorphism. This section presents some papers dealing with the pharmaceutical application of polymorphism appearing since 1969.

One welcome addition to the field of polymorphism is Kuhnert-Brandstätter's book on "Thermomicroscopy in the Analysis of Pharmaceuticals" (34). To show how frequently polymorphism occurs in pharmaceuticals and how numerous the modifications of a substance may be, the author described three representative groups in detail. Of the barbiturates used medicinally, she found that about 70% were polymorphic. A fairly large number of these barbiturates possessed four or five crystalline modifications.

The other groups described were the steroid hormones and sulfonamides; she found 42 polymorphic steroids in the former group and 30 polymorphic sulfonamides and related compounds in the latter group. She also compiled the microscopic behavior of hundreds of pharmaceuticals when subjected to heat and identified the presence of any existing polymorphs.

Another important paper is Buerger's review (35) of the basics of transformation theory and the forms involved in the transformation, namely the polymorphs. He discussed the reasons why some poly-

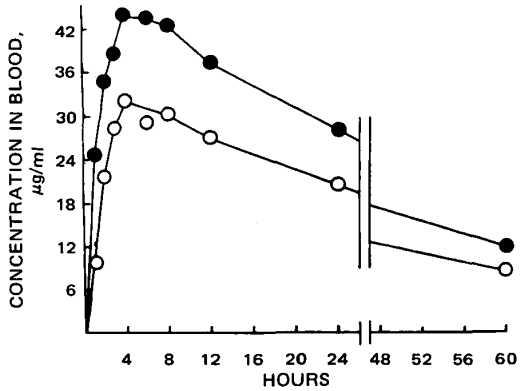


Figure 8—*Mean blood concentration curves of sulfameter crystal Forms II and III. Key: ●, Form II; and ○, Form III. (Reproduced, with permission, from Ref. 49.)*

morphic changes happen faster than others. Kinetic theory predicts that, if the transformation from one polymorphic structure to another is opposed by a potential barrier of magnitude B, the speed of transformation is proportional to $1/e^{B/kT}$.

For some structural changes, when the temperature reaches the transformation temperature, many or all of the atoms can immediately surmount the barrier. Therefore, the transformation is rapid. In other structural changes, few atoms have the thermal energy to surmount the barrier. Appreciable time then is needed for one structure to change to the other through temperature variation; the transformation is relatively sluggish (35).

Examples of Polymorphism—A recent major advance in the field of polymorphism has been the X-ray crystallographer's interest in compounds exhibiting polymorphism. Investigations using single crystals have, for the first time, given the absolute configuration of different compounds exhibiting polymorphism. Perrin and Michel (36, 37) solved the structure of p-chlorophenol where the α-form, the stable modification, is a monoclinic crystal while the β-form is a metastable crystal stable at $-10°$ but also belongs to the monoclinic system. Kahn *et al.* (38), working with cyclohexane, solved the structures of both Phases I and II, the stable phases, but could not solve the structure of Phase III. Phase III is a metastable form, and single crystals are not obtainable.

Of special pharmaceutical importance is the work of Busetta *et al.* on estrone (39). They found that Phases I and II crystallize as orthorhombic crystals while Phase III crystallizes as a monoclinic crystal. This reviewer believes that the method developed by Busetta *et al.*, although tedious, is the ultimate method for establishing whether a polymorph exists and should be used whenever the polymorphism of a compound is in question.

The search for different polymorphs of pharmaceuticals has now spread all over the world, with sulfonamides being extensively studied (40–43).

The p-amino group, the acidic N^1-hydrogen atom, and the oxygens of the sulfonamide group were implicated in the various hydrogen bonding arrangements that distinguish one polymorphic form from another (43). It was postulated that electron-withdrawing and electron-donating groups at the N^1-position influence the strength of the hydrogen bonds formed, hence the tendency of compounds to exist in more than one crystalline form.

The thermal behavior of four crystalline phases of sulfanilamide and of sulfanilamide-d_4 were compared; the deuterated modifications exhibited smaller heats of transition and heats of fusion than the corresponding undeuterated forms (44). Further work was done on the different crystal forms of sulfathiazole (45–47).

The crystal forms of sulfameter were studied and identified as three polymorphs, two solvates, and an amorphous phase (48, 49). The GI absorption of Forms II and III was studied in humans. One gram of either crystal phase was suspended in a mixture of 25 ml each of 20% mucilage of acacia and simple syrup. The mean blood concentration curves are shown in Fig. 8. The energetic crystal Form II had an absorption rate about 1.4 times as great as that of the water-stable Form III. Marketed pharmaceutical preparations of sulfameter were also studied and contained mainly Form III. As a result of the absorption study, the use of Form II, the more biologically available crystal form, was recommended, provided adequate measures are taken to prevent its transformation.

Borka (50, 51), using both differential thermal analysis and IR spectrophotometry, identified the different polymorphs of chloramphenicol palmitate and studied their solid and solution phase transformations. He found that Form B, although metastable, has unusual stability, which, combined with its low solubility in water, assures very reliable drug preparations not only in the dry form as capsules but also when suspended in water.

Aguiar (52) continued his work on the different polymorphs of chloramphenicol palmitate and determined their dilatometric behavior. Aguiar and Zelmer (53) also studied the relative dissolution and solubility of the three polymorphs of chloramphenicol palmitate. From a comparison of the *in vivo* absorption data in humans and thermodynamic parameters, they postulated that when the free energy differences between the polymorphs are high, they might affect the absorption profiles. However, if the free energy differences are not high, as in mefenamic acid, there are no significant differences in their absorptions as measured by blood levels.

Banerjee *et al.* (54) followed the absorption of amorphous and polymorph A chloramphenicol palmitate suspensions and found that the absorption of the amorphous form was "definitely superior" in children. Miyamoto *et al.* (55) also studied the differential scanning calorimetry of chloramphenicol palmitate and its phase transitional behavior during the preparation of its oral suspension.

Finally, after billions of aspirin tablets have been produced and consumed, Tawashi (56) reported that aspirin is dimorphic. He also studied the GI absorption of these two forms in humans; 600 mg of each phase dispersed in water was ingested by each volun-

teer and the total serum salicylates were measured. The salicylate concentration–time curve is given in Fig. 9 (57). The disclosure by Tawashi generated one of the most concentrated efforts in the field of polymorphism, and the outcome is yet far from the fate of Derjaguin's "polywater."

Summers *et al.* (58) agreed that aspirin is polymorphic and proposed six different forms; Borka (59), using data obtained from solution phase transformations, agreed that aspirin is at least dimorphic. The major opponents to the polymorphism of aspirin have been Mitchell and coworkers (60, 61), who suggested that differences seen in different aspirins crystallized from different solvents are due to crystal defects (60), aging and ripening of the crystals, or poisoning by impurities other than salicylic acid (61).

Questions (62–64) raised on crystal habits, crystal size, or salicylic acid impurities, coupled with the mentioned controversies, will surely generate more work on the crystal chemistry of aspirin until a final agreement is reached. This author believes that the best way to solve the questions will be by doing a total structure determination using single-crystal X-ray crystallography, which has already been successfully used with other polymorphic substances.

Other compounds reported to exhibit polymorphism are water with nine polymorphs (65), sulfur with 12 crystalline phases (66), chlordiazepoxide hydrochloride (6), methylchloromethane (67), cholesteryl palmitate (68), adiphenine hydrochloride (69); benzocaine picrate (70), phenobarbital (71), meprobamate (72), and erythromycin free base (73). The polymorphism of 3-(3-hydroxy-3-methylbutylamino)-5-methyl-*as*-triazino[5,6-*b*]indole (74) and 1-(2,3-dihydro-5-methoxybenzo[*b*]furan-2-ylmethyl)-4-(*o*-methoxyphenyl)piperazine hydrochloride (75) also was reported.

Dissolution Kinetics of Polymorphs—Kuhnert-Brandstätter and Burger (76–78) developed mathematical derivations on dissolution kinetics of both crystalline and amorphous phases of drugs and applied them to crystalline sulfathiourea and pyrithyldione and to crystalline and amorphous phases of calcium D-pantothenate. Dissolution studies were also carried on with different polymorphs of phenobarbital (79). Phase transitions between different crystalline forms were followed for methylprednisolone in the solid form (80), barbital (81), and sulfameter (sulfamethoxydiazine) (82) suspended in water.

Dissolution studies were taken one step further with the development of a mathematical derivation to determine the transition temperature of sulfathiazole polymorphs and the transition temperature between hydrate and anhydrous forms of phenobarbital by measuring their initial dissolution rates (83, 84). This method was in good agreement with values obtained by the conventional solubility equilibrium method.

Pharmaceutical Application of Polymorphism—*Polymorphism and Tableting Behavior of Powders*—Simmons *et al.* (85) reported that tolbutamide exists as Forms A and B, and they evaluated their respective tableting behavior. A preliminary evaluation on a rotary press revealed that Form B was responsible for both powder bridging in the hopper and extensive capping problems during tableting. This behavior was due to the platy habit of Form B and could be corrected by using nonplaty Form A raw material.

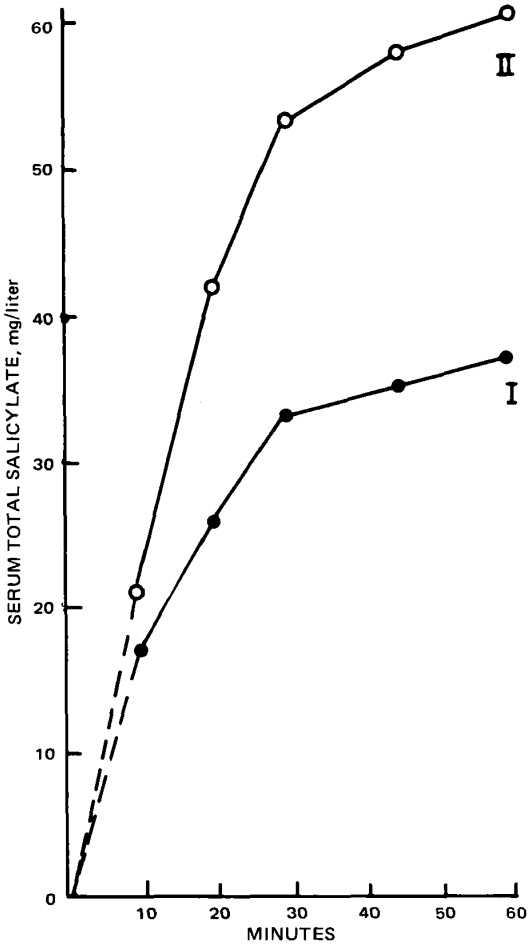


Figure 9—*Serum total salicylate concentration after oral ingestion of aspirin Forms I and II. (Reproduced, with permission, from Ref. 57.)*

Polymorphism and Physically Stable Dosage Forms—Pearson and Varney (86) followed the growth of oxyclozanide crystals in quiescent suspensions with the use of a particle counter[1]. They found that there was an increase in the particle size as a result of an isothermal, solvent-mediated phase transition between two polymorphs. The rearrangement of molecules in the crystal occurs through selective dissolution and redeposition between crystals of different chemical potential. By this mechanism, the less soluble phase grows at the expense of the more soluble phase. To form a stable suspension, the initial use of a less soluble polymorph is mandatory. However, as Ebian *et al.* (87) reported in their work with sulfameter (sulfamethoxydiazine), the addition of transformation-retardation agents, *e.g.*, polyvinylpyrrolidone, might be useful.

[1] Coulter.

Polymorphism and Chemical Stability—Munshi and Simonelli (88), working with two polymorphs of methylprednisolone, found that Phase II degraded when exposed to various temperatures and relative humidity while Phase I was stable. They also found that this surface reaction was catalyzed by heat, UV light, and humidity.

As a result of the scientific evaluation of different polymorphs of drugs, there is a totally new awareness in this field. This awareness was demonstrated with the introduction of a new section on X-ray diffraction in NF XIII (89) and special X-ray diffraction patterns for different polymorphs of some drugs being included in the compendia. It is encouraging that this awareness of different polymorphs of different chemicals is now reflected in drug catalogs (90). This author's hope is that this kind of information will be available for any marketed chemical.

Molecular adducts of chemicals and the solvent of crystallization can be in either a stoichiometric proportion, as in the case of solvates, or a nonstoichiometric proportion, as in the case of inclusion compounds.

STOICHIOMETRIC ADDUCTS-SOLVATES

Definition—During crystallization from a solution, crystals separating may consist of a pure component or be a molecular compound. Molecular compounds may contain two or more constituents that have completely satisfied classical "valence forces" and are crystallized together as a new single crystalline entity. Solvates are molecular complexes that have incorporated the crystallizing solvent molecule in their lattice. When the solvent incorporated in the solvate is water, it is called a hydrate.

To distinguish solvates from polymorphs, which are not molecular compounds, the term pseudopolymorph is used (91). All solvates are formed with stoichiometric proportions between the compound and solvent of crystallization (92). Just as different chemical compounds can have different polymorphs, solvates of different compounds can exhibit polymorphism too. Kuhnert-Brandstätter and Gasser (91), in their work with fluocortolone, found dimorphism of the ethanolic solvates. Similarly, polymorphism was also reported for hydrates of fluprednisolone (29) and succinylsulfathiazole (93).

Examples of Solvates—The pharmaceutical literature on examples of solvates is relatively new, but an extensive amount has been published in the last 20 years. Kuhnert-Brandstätter and coworkers (91, 92, 94–96) worked extensively on solvates of steroids and found that solvate formation is independent of polymorphism. Of all the steroids examined, they found that estradiol formed solvates with all 30 solvents tested, the highest number of solvates so far recorded (92). Other steroids that have been observed to form solvates include hydrocortisone acetate (97), cortisone acetate (98), fluprednisolone (29), the *tert*-butylacetates of prednisolone and hydrocortisone (99), and fludrocortisone (fluorohydrocortisone) acetate and cholesterol (93).

Many antibiotic–antibacterials can also form solvates, *e.g.*, erythromycin (100), gramicidin (101), furaltadone (nitrofurmethone) (102), ampicillin (103, 104), cephaloridine (105), chloramphenicol (106), griseofulvin (107), sulfanilamide (108), sulfabenzamide (43), sulfaguanidine (43), succinylsulfathiazole (93), sulfameter (sulfamethoxydiazine and sulfamonomethoxine) (48, 109). Other miscellaneous compounds also forming solvates include caffeine, theophylline, glutethimide (93), ouabain (110), phenobarbital (111), triazinoindoles (74, 112), and at least 90 other hydrates included in USP XVIII (32) and NF XIII (89).

Preparation of Solvates—Most solvates are prepared by crystallization of the solvated compound from the respective solvent of solvation. In some cases where mixed solvents are used, solvates might be formed from either or both solvents. Holden and Singer (113), in their book on crystal growing, discussed salt hydrate formation where a salt is added to water which weakens the electric forces between the ions and also attracts the ions away from the solid. Once ions are free, they gather water molecules around them. Positive ions gather water molecules to them more readily than negative ions. When these hydrated ions arrive at the surface of a growing crystal, they crystallize into a salt hydrate, carrying some or all of their attached water molecules into the solid composition (Fig. 10).

Even when the number of water molecules is definite and the hydrated ions are combined into a crystalline solid (an orderly arrangement of ions and water molecules), they may, nonetheless, be able to combine in several ways to form different solids. For each hydrate, the number of water molecules per ion

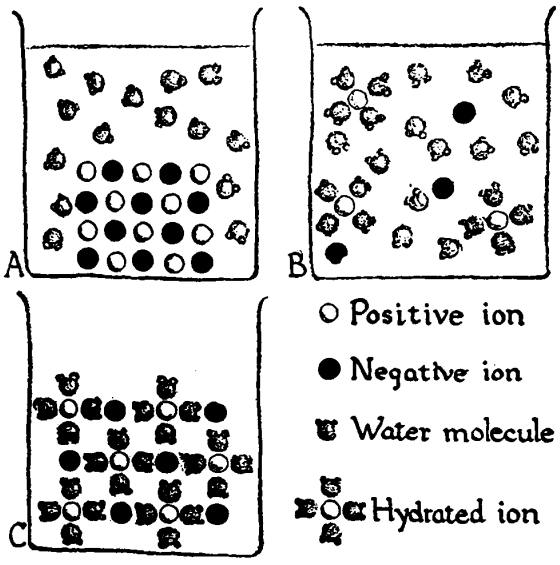


Figure 10—*Formation of a salt hydrate. When the anhydrous salt comes in the presence of water (A), it dissolves and the positive ions become hydrated (B). When crystallizing from solution again, the ions may remain hydrated and then the water molecules play an orderly role in the crystalline salt hydrate (C). (Reproduced, with permission, from Ref. 113.)*

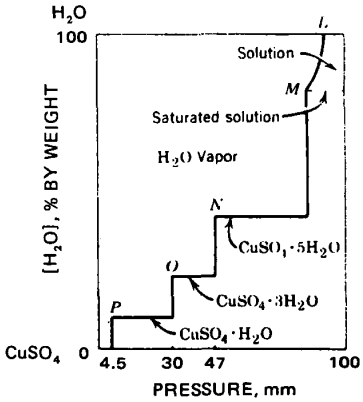


Figure 11—*Pressure–concentration curve for the cupric sulfate–water system at 50°. (Reproduced, with permission, from Ref. 114.)*

has a definite value. Solvates of a compound with different moles of solvent can be prepared in different ways, either by holding the temperature constant or by maintaining the pressure constant in the concentration–temperature–pressure systems.

Figure 11 represents the concentration–pressure curve at 50° for the cupric sulfate–water system (114). If a dilute solution of cupric sulfate is placed in an evacuated desiccator at 50° and water vapor is removed so slowly that an equilibrium is maintained, the water vapor pressure changes along the *LMNOP* line. As water is removed, the cupric sulfate concentration increases; the vapor pressure of the solution then decreases along line *LM*. When the solution becomes saturated, the pressure remains constant (vertical line at *M*), while the water is removed.

The gross composition of the system changes as the water is removed, and the relative amounts of saturated solution and $CuSO_4 \cdot 5H_2O$ change. However, since the compositions of the two phases do not change, the vapor pressure remains constant. When all of the solution has disappeared, the pressure drops abruptly to 47 mm at *N*. The pressure over a $CuSO_4 \cdot 5H_2O$ and $CuSO_4 \cdot 3H_2O$ mixture remains constant at 47 mm. When all the $CuSO_4 \cdot 5H_2O$ has been dehydrated to $CuSO_4 \cdot 3H_2O$, the partial pressure of water drops abruptly to 30 mm at *O*. It remains constant until 2 more moles of water/mole of cupric sulfate is lost at *P*. Then the water vapor pressure drops to 4.5 mm and remains constant until dehydration is complete (114).

Table II—Water Content and Temperature of Isolation of Ouabain Hydrates

Hydrate	Temperature of Isolation
Ouabain·$9H_2O$	0–15°
Ouabain·$8H_2O$	15–28°
Ouabain·$4\frac{1}{2}H_2O$	20–30° [a]
Ouabain·$4H_2O$	20–30° [b]
Ouabain·$3H_2O$	20–30° [c]
Ouabain·$2H_2O$	28–90°
Ouabain	150° [d]

[a] From 95% ethanol. [b] From 97% methanol. [c] From dioxane containing 6% water. [d] Obtained by heating ouabain recrystallized from ethanol in abderhalden apparatus at 150°.

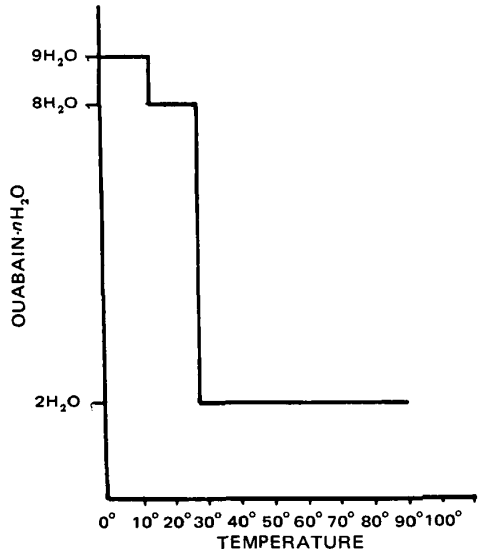


Figure 12—*Temperature–water composition phase diagram for ouabain hydrates. (Reproduced, with permission, from Ref. 110.)*

Trivedi *et al.* (110), using the alternative method of holding pressure constant, obtained hydrates of ouabain with different molecules of water by adding excess amounts of ouabain to the solvent for recrystallization and heating it above the crystallization temperature. The solutions were then allowed to cool to the temperature desired for crystallization. After a sufficient amount of ouabain had crystallized, the containers were closed to help equilibrate the system without evaporation. In this manner, ouabain was crystallized at 0° and at intervals of 10° from 20 through 90°. Six different ouabain hydrates were obtained, depending on the temperature of crystallization. Table II shows the water content and tempera-

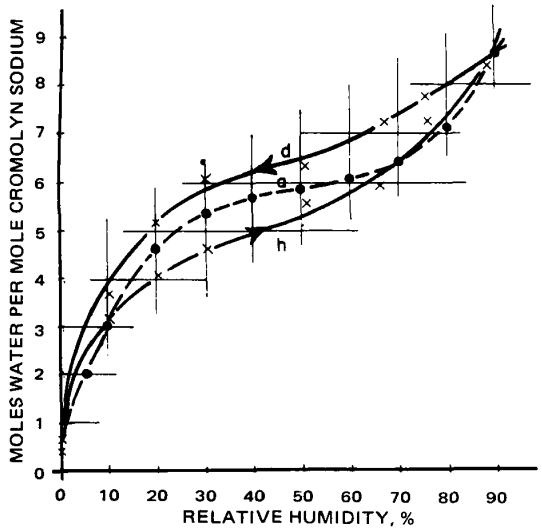


Figure 13—*Water content as a function of relative humidity during hydration (h) and dehydration (d), and an average curve for all data determined (a). (Reproduced, with permission, from Ref. 115.)*

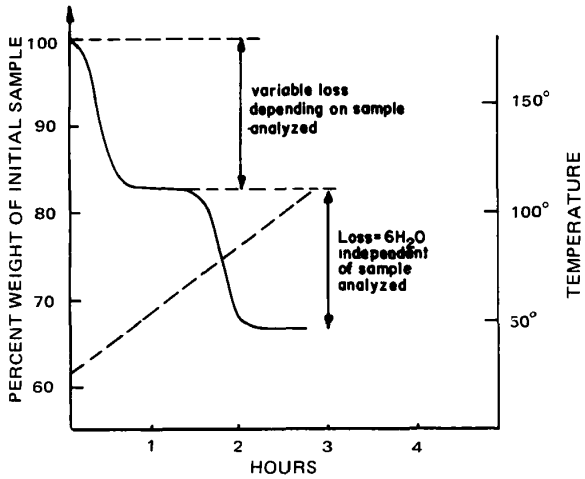


Figure 14—*Thermogravimetric analysis thermogram of tetracycline base with a linear increase in temperature. (Reproduced, with permission, from Ref. 116.)*

ture of isolation. The temperature–water composition phase diagram for the hydrates isolated from water is illustrated in Fig. 12.

Cox *et al.* (115), in their work on solid-state chemistry of cromolyn sodium, obtained a continuous series of interstitial solid solutions or lyotropic mesophases with water by changing the relative humidity. Figure 13 represents the change of water content as a function of relative humidity. The hydration curve shows that, although there is a region of slow increase of moisture content with increasing humidity, there are no sharp plateaus corresponding to fixed hydrates. This absorbed water, amounting to as much as 24 wt. % at about 90% relative humidity, resulted in reversible expansion of the lattice, especially along

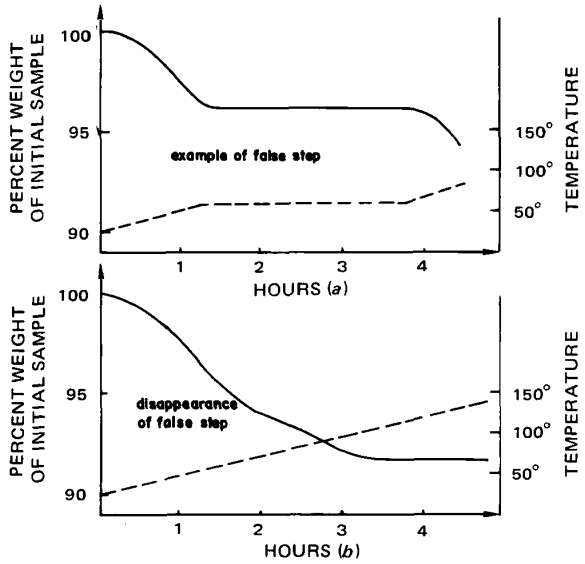


Figure 15—*Thermogravimetric analysis thermogram of SD-1223-01. Key:* a, *using a constant temperature of 60°; and* b, *with a linear increase in temperature. (Reproduced, with permission, from Ref. 116.)*

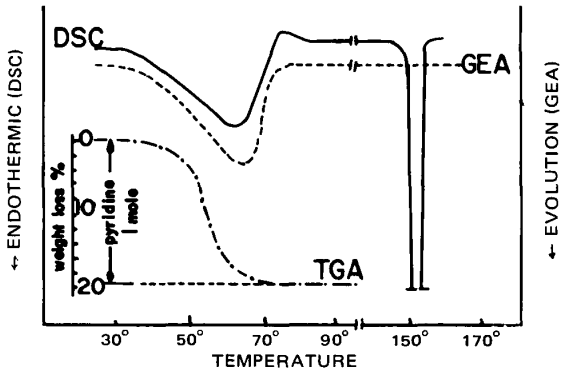


Figure 16—*Differential scanning calorimetry–gas evolution analysis and thermogravimetric analysis thermogram of chloramphenicol pyridinate. (Reproduced, with permission, from Ref. 106.)*

the *b* crystallographic direction. This, in turn, caused changes in X-ray diffraction patterns, density, and other physical properties.

Characterization of Solvates—Different analytical methods have been used for identification of either the solvates or the solvent of the molecular complex.

Methods Used to Identify Solvates—Hirtz *et al.* (116), using thermogravimetric analysis, studied the solvation of organic compounds with pharmaceutical interest. Thermograms of different solvates show different steps of desolvation, which correspond, at a certain interval of temperature and pressure, to the stable solvate. The distinction of the steps on the thermogram could be due to real solvation or to pseudosolvation, which is due to the special setup of the experiment.

Real solvation for tetracycline base is illustrated in Fig. 14. A linear increase of temperature produces a loss of initial weight, which varies from sample to sample and is due to volatilization of "free water." This loss is followed with a second loss of weight, in this case equivalent to 6 moles of water and independent of sample analyzed, indicating that the original sample was a hexahydrate. In the case of thermograms that are the results of special sets of experimental conditions, one can see, as in Fig. 15, that when the temperature increase is not linear, a false step develops on the thermogram which disappears during a linear temperature increase.

Using differential thermal analysis, Brancone and Ferrari (109) were able to demonstrate that sulfameter is a solvate. Similarly, Sekiguchi *et al.* (107) used differential thermal analysis to identify different hydrates of phenobarbital, theophylline, and other organic pharmaceuticals.

Differential scanning calorimetry, a method similar to differential thermal analysis, was also used by Sekiguchi *et al.* (107) to identify the chloroform solvate of griseofulvin.

Gas evolution analysis can be done simultaneously with differential scanning calorimetry. Himuro *et al.* (106), in their work on pyridine solvate of chloramphenicol, plotted differential scanning calorimetry–

gas evolution analysis and thermogravimetric analysis thermograms (Fig. 16). There is a very close correlation of the three different methods used in identifying the solvate.

Trivedi *et al.* (110), using the petrographic microscope, determined optical crystallographic data for ouabain hydrate. These data included the crystal system, optic orientation, optic sign, axial angle, dispersion, common orientation, and refractive indexes. Kuhnert-Brandstätter and Grimm (95) used thermomicroscopy to identify different solvates and polymorphic modifications of different steroids.

Most X-ray diffraction work reported employed powder diffraction. This method was used to differentiate fluprednisolone polymorphs from fluprednisolone solvates (29), to study pseudopolymorphism of furaltadone (102), and to differentiate chloramphenicol from its pyridinate solvate (106). Moustafa *et al.* (48) used powder diffraction to differentiate polymorphs and solvates of sulfameter. If the purity of a sample is established, powder X-ray diffraction is one of the best methods available to differentiate any crystalline modification, be it polymorphs, solvates, or clathrates. Busetta *et al.* (117), continuing their work on single-crystal X-ray crystallography of pharmaceuticals, determined the crystal structure of methanol plus water, of ethanol, and of dimethyl sulfoxide solvates of diethylstilbestrol. They found that all three phases are triclinic crystals. Single-crystal X-ray diffraction also was used to determine the structure of different pharmaceutical hydrates (118, 119).

IR spectrophotometry is much more useful for distinguishing between solvates and anhydrous forms than for identifying polymorphs because of the addition of new stretching frequencies resulting from solvation. Biles (99) used IR spectrophotometry to differentiate crystalline modifications of the *tert*-butylacetates of prednisolone and hydrocortisone. Haleblian *et al.* (29) used IR spectrophotometry in their work on characterization of different solid phases of fluprednisolone. Kuhnert-Brandstätter and Grimm (94, 95) also used this method for identification of solvates of some of the different steroids studied, although they felt that it was difficult and often impossible to distinguish crystal forms containing solvent from polymorphic modifications (94).

Methods Used to Identify Solvent of Solvation—Most solvents of solvation are determined by using some method that produces the release of the solvent, which, in turn, is trapped and identified. If the solvent of solvation is water, *e.g.*, hydrates, some of the USP XVIII (120) methods for water determination can be used. Of these, the most important is the Karl Fischer titrimetric method. This method was used to identify the hydrates of fluprednisolone (29) and ouabain (110).

IR spectrophotometry was used to identify the solvents of solvation that are readily vaporized and give satisfactory spectra at low concentrations in conventional gas cells (121). The sample was heated in polyethylene glycol 300, a liquid of high boiling point, which dissolves the sample, and the vapor was examined in an evacuated gas cell for the presence of absorption spectra characteristic of a particular solvent.

The GC method described in the British Pharmacopoeia 1968 was also used to identify solvents (121).

Investigation of hydrated crystals using proton magnetic resonance was pioneered by Pake (122) and advanced by McGrath and Silvidi (123) and Pedersen (124). Chapman *et al.* (105), in their work on cephaloridine, obtained quantitative information on the different solvated solvents by using proton magnetic resonance.

Differential scanning calorimetry has also been used. Different amounts of water in cortisone acetate hydrates were measured by calibrating the effluent analyzer, using $CuSO_4 \cdot 5H_2O$ as the standard (98).

Properties of Solvates—*Dissolution Rate of Solvates*—Shefter and Higuchi (93), in their work on the dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals, were the first to treat mathematically the dissolution rate of solvates. The dissolution of a solvate can be expressed in water as in Scheme II:

$$A{:}nB_{\text{solid}} \rightleftharpoons A_{\text{aqueous}} + nB_{\text{aqueous}}$$

Scheme II

where A is the drug component and B is the solvent that is solvated. The rate of dissolution can be given by:

$$G = \frac{dC_A^*}{dt} = kD_A(C_A - C_A^*) = \frac{1}{n}\frac{dC_B^*}{dt} = \frac{kD_B}{n}(C_B - C_B^*) \quad \text{(Eq. 2)}$$

where C_A^* and C_B^* are the concentrations of the two species in the bulk of the solution, C_A and C_B are their respective concentrations at the crystal surface, D_A and D_B are the respective diffusivities, and k is a combined geometric and agitation factor.

For the case where $n = 1$ and $D_A = D_B$, Eq. 2 can be simplied to:

$$G = \frac{dC_A^*}{dt} = kD_A(\sqrt{Ksp} - C_A^*) \quad \text{(Eq. 3)}$$

where $[C_A][C_B]^n = Ksp$.

From Eq. 3, it can be seen that C_A^* may be able to build above the solubility of A itself in water. If the concentration of C_B^* is increased much more than C_A^* by the addition of the solvating solvent in the dissolution medium, then:

$$G = \frac{KD_A Ksp - kD_A C_A^*}{C_B^*} \quad \text{(Eq. 4)}$$

which indicates that the dissolution rate for the solvate will decrease with an increase of B in solution.

Nogami *et al.* (111), in their work on the dissolution of organic medicinals involving simultaneous phase changes, defined dissolution rate as:

$$\frac{dC}{dt} = k_t\{C_{SA}e^{-k_t t} + C_{SH}[1 - e^{-k_r t}] - C\} \quad \text{(Eq. 5)}$$

where C is the concentration in the bulk liquid, C_{SA}

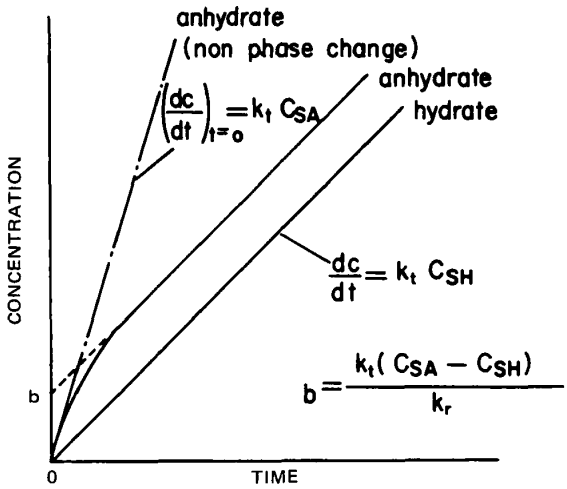


Figure 17—*Schematic illustration for relationship between concentration and time in initial time of dissolution according to Eqs. 8 and 10. (Reproduced, with permission, from Ref. 111.)*

is the saturated concentration of the anhydrous phase, C_{SH} is the saturated concentration of the hydrate, and k_r and k_t are the rate constants of crystallization and transport processes, respectively. Since $C = 0$ and $t = 0$, Eq. 5 is integrated to:

$$C = \frac{k_t(C_{SA} - C_{SH})}{k_t - k_r}(e^{-k_r t} - e^{-k_t t}) + C_{SH}(1 - e^{-k_t t}) \quad \text{(Eq. 6)}$$

This equation expresses the general dissolution curve involving the phase change caused by hydration. Since C_i, the effective concentration on the solid surface, is initially greater than C, Eq. 5 is modified to:

$$\frac{dC}{dt} = k_t(C_{SA}e^{-k_r t} + C_{SH}[1 - e^{-k_r t}]) \quad \text{(Eq. 7)}$$

Under the initial condition $C = 0$ at $t = 0$, Eq. 7 is integrated to yield:

$$C = \frac{k_t(C_{SA} - C_{SH})}{k_r}[1 - e^{-k_r t}] + k_t C_{SH} t \quad \text{(Eq. 8)}$$

Since t may be sufficiently long, even in the initial stage, Eq. 8 gives a linear relationship with the gradient of $k_t C_{SA}$. Then, when the linear portion of Eq. 8 is extrapolated, the intercept, b, is given by:

$$b = \frac{k_t(C_{SA} - C_{SH})}{k_r} \quad \text{(Eq. 9)}$$

Equation 9 predicts how much the dissolution data of the anhydrous phase undergoing phase transition deviate from the dissolution curve expected from the hydrate alone.

The initial dissolution rate, $(dC/dt)_t = 0$, is derived from Eq. 7 with:

$$\left(\frac{dC}{dt}\right)_{t=0} = k_t C_{SA} \quad \text{(Eq. 10)}$$

which corresponds to the dissolution rate of the anhydrous phase undergoing no phase transition. The general patterns of dissolution behavior according to Eqs. 8 and 10 are illustrated in Fig. 17 (111).

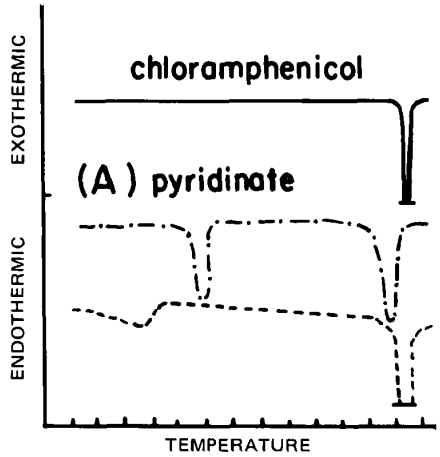


Figure 18—*Differential scanning calorimetry thermogram of chloramphenicol pyridinate solvate. (Reproduced, with permission, from Ref. 106.)*

The rate of dissolution of fluprednisolone above a certain temperature may be faster than the rate of transition (anhydrous → hydrous) and not noticeable in the concentration *versus* time plots (125). Ways of calculating different thermodynamic parameters were derived for the anhydrous–hydrated systems, *e.g.*, enthalpy of hydration, free energy of hydration, entropy change, and transition temperatures (93).

Melting Behavior of Solvates—Clear solvate crystals, when observed by transmitted light under a hot stage microscope, lose their solvent of crystallization and become either cloudy or opaque crystals, a process known as pseudomorphosis. Sometimes a solvate first melts and then resolidifies as the temperature rises to give the solvate-free form, which finally again melts. This phenomenon is illustrated by the differential scanning calorimetry curves of chloramphenicol pyridinate (Fig. 18) (106). The differential scanning calorimetry curve consists of three peaks (Fig. 18) (106):

1. The first endothermic peak is due to the desolvation of pyridine, because the shape and the temperature of peak decline are strongly influenced by experimental conditions.
2. The small exothermic rise is due to crystallization of amorphous solid or a metastable melt.
3. The second sharp endothermic peak represents melting of desolvated chloramphenicol.

Brancone and Ferrari (109) found, using differential thermal analysis, that the shallow endotherm of sulfameter at 120° is due to a solvent. They confirmed this observation by running another thermogram under reduced pressure where the endotherm, due to the solvent, had shifted to 66°.

A classical method for distinguishing solvates from polymorphs involves observation of the melting behavior of crystals embedded in silicone oil, where, upon heating, bubbles of solvent are generated by solvates. In the case of polymorphs, no such generation occurs.

This reviewer's opinion is that during heating of crystals embedded in silicone oil, the temperature at

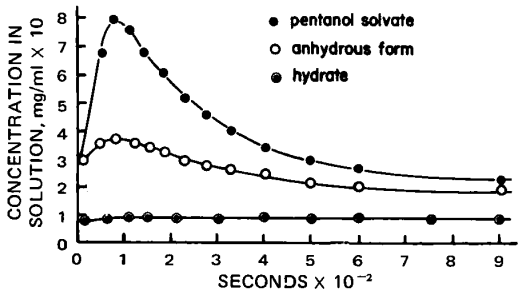


Figure 19—*Dissolution behavior of different forms of succinylsulfathiazole in ~0.001 N sulfuric acid solution at 20°. (Reproduced, with permission, from Ref. 93.)*

which gas bubbles generate is in the vicinity of the boiling point of the solvating solvent. In the case of clathrates (discussed later), this temperature is much higher than the boiling point of the clathrated solvent and very near the melting point of the clathrate.

Pharmaceutical Application of Solvates—*Dissolution*—As anticipated from the theoretical properties of solvates, the dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals agreed with the theory (93). Figure 19 shows the dissolution behavior of anhydrous, hydrated, and pentanol-solvated forms of succinylsulfathiazole. These curves confirm the theoretical prediction that the dissolution rate of a solvate may be many times greater than the anhydrous form. On the other hand, the dissolution rate of the hydrate is less than the anhydrous form.

Another interesting confirmation of theory (93) is the effect of the concentration of the solvating solvent in the dissolution medium. When measuring the dissolution of the pentanol solvate of fludrocortisone acetate, the dissolution rate was retarded by the addition of pentanol in the dissolution medium. According to this study, it is conceivable that solvates will find broad use in dosage form development "due to their higher temporary solution concentrations and rates of solution which can be obtained by their use instead of the anhydrous form. This is because the system utilizes the free energy of dilution of the complexing agent to raise the solubility of the drug." (93).

Bioavailability—The physicochemical factors influencing the absorption of the anhydrous and trihydrate forms of ampicillin were studied (103). The aqueous solubility of the anhydrous form was 20% greater than the trihydrate at 37° (10 and 8 mg/ml, respectively). The effect of the observed solubility differences on the *in vitro* availability of the drug was also studied. The T_{50} (time required for 50% of the labeled amount to appear in solution) was 7.5 and 45 min for the anhydrous and trihydrate forms, respectively.

In vivo experiments were also done with dogs and humans, where the anhydrous and the trihydrate forms of the drug were given as oral suspensions or capsules. The anhydrous form produced higher and earlier peaks in the blood serum than the trihydrate form. This result was more pronounced in the suspension formulations. Figure 20 shows the mean blood serum concentration in human subjects after oral administration of a 250-mg dose of the suspension. With both formulations, the area under the blood serum level *versus* time curve was greater with the anhydrous form, indicating that the anhydrous form is more efficiently absorbed.

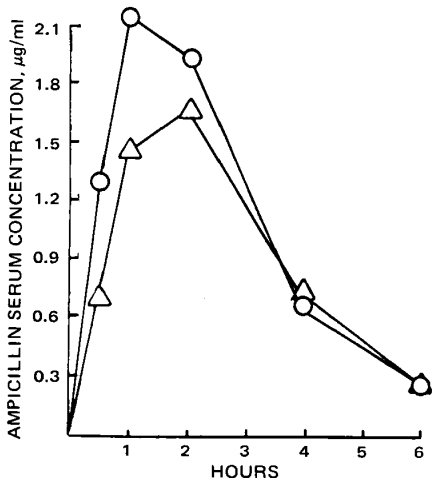


Figure 20—*Mean blood serum concentrations of ampicillin in human subjects after oral administration of 250-mg doses of oral suspension. Key: ○, anhydrous; and △, trihydrate. (Reproduced, with permission, from Ref. 103.)*

Hill *et al.* (126) questioned the conclusion (103) that the differences seen in ampicillin bioavailability were due to the hydration of ampicillin, since they found that the solubilities of the two phases in dilute hydrochloric acid at 37° were very similar. As a result of their data, they suggested that differences in the bioavailability are related to formulation factors rather than the hydration state of the raw material.

Ballard and Biles (127) studied the *in vitro* absorption rates of the *tert*-butylacetates of hydrocortisone and prednisolone and their solvates by the pellet implantation technique. They found that solvates affect the absorption rate from solid drug implants (Table III). With prednisolone tebutate pellets, for example, the monoethanol solvate (Phase II) had an absorption rate 4.7 times greater than that of the anhydrous phase (Phase I). The hemiacetone solvate (Phase IV) had practically the same mean absorption rate as the

Table III—Absorption Rate of the *tert*-Butylacetates of Hydrocortisone and Prednisolone

	Crystal Modification	Absorption Rate per Unit Area, mg/hr/cm²
	Prednisolone *tert*-Butylacetates	
Phase I	Anhydrous phase	1.84×10^{-3}
Phase II	Monoethanol solvate	8.70×10^{-3}
Phase III	Hemiacetone solvate	2.20×10^{-3}
	Hydrocortisone *tert*-Butylacetates	
Phase I	Monoethanol solvate	1.83×10^{-3}
Phase II	Monoethanol solvate	7.32×10^{-3}
Phase III	Hemichloroform solvate	7.40×10^{-3}
Phase IV	Anhydrous	4.74×10^{-3}

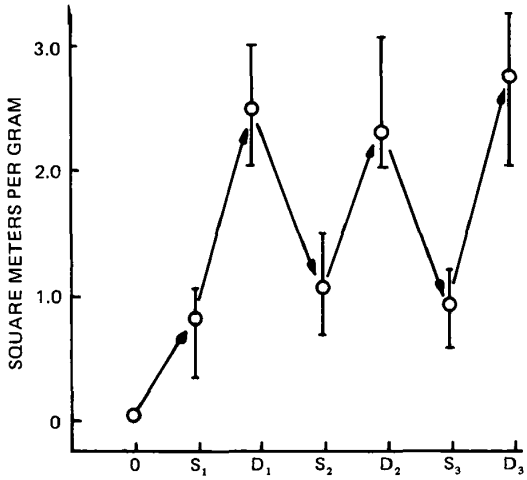


Figure 21—*Effect of repeated solvation and desolvation upon specific surface area of griseofulvin and its chloroformate. Key: O, ordinary crystal; S_1, solvated crystal; and D_1, desolvated crystal. (Reproduced, with permission, from Ref. 107.)*

anhydrous phase. With hydrocortisone *tert*-butylacetate pellets, the mean absorption rate for the solvates were all significantly different from the mean rate of the anhydrous phase (Phase IV). Also one monoethanol solvate (Phase II) had 4.0 times the mean absorption rate of the other monoethanol solvates (Phase I). These results indicate that drug solvates may exhibit dimorphism and each form may exhibit different *in vitro* absorption rates.

In a comparison of the physical properties and biological activities of some crystalline phases of fluprednisolone, Haleblian *et al.* (125) isolated one amorphous and six crystalline phases. Two phases were dimorphic solvates containing 1 mole of water. One existed as the *tert*-butylamine disolvate and three were anhydrous trimorphs. Using the Wruble apparatus, they determined the *in vitro* aqueous dissolution rates (6 rpm and 23°) of the anhydrous and hydrated forms. The dissolution rate ratio for the highest and lowest energetic phases was 2.24, where Form I was the most energetic phase and the α-monohydrate was the least energetic phase.

Similar results were obtained by comparing the *in vivo* dissolution rates of pellets of each crystalline phase, implanted subcutaneously by the method of Ballard and Nelson (128) into males rats. The *in vivo* dissolution rate ratio between the highest and lowest energetic phases was 1.61. Thus, the biological uptake of Form I of fluprednisolone in pellet form was 1.61 times that of the α-monohydrate phase. This value illustrates the effect that crystalline phases may have on biological activity. The *in vivo* and *in vitro* dissolution rates were also correlated with a pharmacological response–adrenal cortex atrophy of the rats. The adrenal cortex atrophy resulting from the uptake of Form I from pellet implants was 1.46 times that of the α-monohydrate.

Physically Stable Suspensions—When aqueous topical suspensions of two anhydrous forms of two different triazinoindoles were formulated, both produced large crystals upon aging. When these crystals were isolated, both were found to be monohydrates of the respective compounds. When the air-milled monohydrate of triazinoindole I, which is labile but does not lose water during milling, was used, suspensions resulted which were physically stable for 2 years.

Table IV—Effect of 3 hr of Exposure to Direct Sunlight or 1 hr of Exposure to 85° of Different Crystals of Coenzyme Form Vitamin B_{12} (Average of Two Runs)

	Percent Remaining	
	After 3 hr of Exposure to Direct Sunlight	After 1 hr of Exposure to 85°
Conventional crystals	74.85	23.70
Hydrated Form A	98.75	72.70
Hydrated Form B	73.75	37.25

Particle-Size Reduction of Medicinal Compounds—Sekiguchi and coworkers (106, 107, 129, 130), working with griseofulvin and chloramphenicol, developed a method of crystal-size reduction involving phase conversion. The technique required use of a proper solvent which forms a solvate with a medicinal compound and which also can be easily desolvated. The effects of repeated solvation and desolvation on the specific surface area of griseofulvin and its chloroformate particles were examined using the Brunauer, Emmett, and Teller (131) method with nitrogen as the adsorbing gas. When ordinary crystals were sorbed with chloroform vapor under vacuum, the apparent volume of the crystal mass was increased by about 50%. But continued desorption and sorption did not change the volume.

A zigzag curve was obtained, and the size reduction was practically finished by one cycle of solvation and desolvation (Fig. 21) (107).

The specific surface area after each desolvation exceeded 2 m^2/g; that of the solvated crystals was about 1 m^2/g after the second solvation. Although no liquid was observed visually at the end of sorption of vaporous chloroform, a minute amount of liquid probably was formed between particles by such a capillary condensation. Therefore, the primary particles are bridged, so surface area is decreased considerably. It was concluded that repeated applications of the method influence the specific surface area only slightly. However, when a solid substance forms a molecular compound with a gas, its surface area increases continuously by repetition of sorption and desorption of the gas (107).

Solvates and Chemical Stability—A patent was granted (132) for light- and heat-stable hydrate crystals of the coenzyme form of vitamin B_{12}. The coenzyme form of vitamin B_{12} can exist as conventional crystals (not specified if anhydrous or not) and as two hydrates, A and B, containing 15% weight of water. Table IV shows the effect of 3 hr of exposure to direct sunlight and also of 1 hr of exposure to 85°. From these data, it can be seen that the hydrate form A is chemically more stable than the other two forms.

Hydrocortisone 21-*tert*-butylacetate can spontaneously oxidize in air and change from 11β-ols to 11-

ones (133). This reaction requires molecular oxygen and produces water. The reaction is accelerated by heat and greatly accelerated by free-radical initiators or UV light. Three crystalline phases of the compound were studied. Phase A is a form in which the crystallizing solvent is held within the crystal in nonstoichiometric proportion; it can be completely desolvated in a vacuum without changes in the X-ray diffraction pattern. Phase B is a stoichiometric solvate. Desolvation is accomplished only at elevated temperature and is accompanied by changes in the X-ray diffraction pattern. Phase C is a nonsolvated form. Esters subject to air oxidation are Phase A, while Phases B and C are resistant to oxidation. Prednisolone tebutate behaved similarly to a lesser degree.

Inclusion compounds are a specialized form of molecular compounds; they do not exhibit stoichiometry. The title "inclusion compounds" for this class of complexes was first used by Schlenk (134, 135). Inclusion compounds vary from the other molecular compounds in that hydrogen bonding plays a minor role or is even nonexistent (136). In all three general types of inclusion compounds, a guest molecule is physically entrapped in a host molecule. The constituents are usually present in constant, but not necessarily stoichiometric, proportions.

The formation of these inclusion compounds[2] or adducts are dependent on the respective molecular size and shape of the constituents. The hollow space in which the guest molecule is enclosed may be a channel, a layer, or a cage.

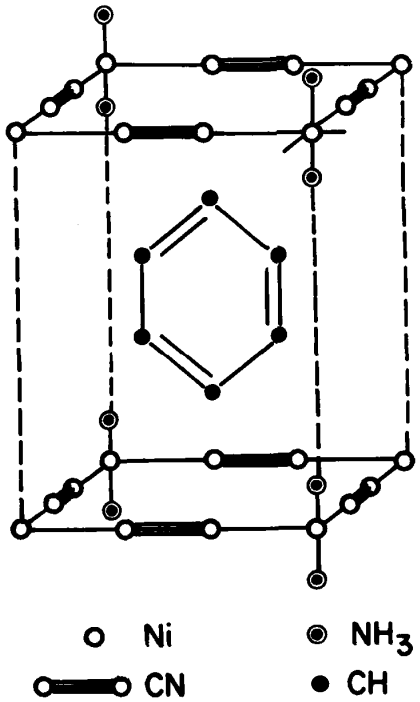


Figure 22—*Schematic representation of the clathrate of benzene with an ammonia-nickel cyanide complex [$Ni(CN)_2NH_3$—C_6H_6]. [Reproduced, with permission, from* Nature, ***163****, 566(1949).]*

NONSTOICHIOMETRIC ADDUCTS-CLATHRATES

Definition—Clathrates are inclusion compounds. They were given this name by Powell (138) since the guest is enclosed or protected by crossbars of a grating. According to one source (139), a clathrate is a single-phased solid with two distinct components: the host and the guest. The guest is retained in closed cavities provided by the crystalline structure of the host. Generally, a cage and its enclosed molecule(s) are taken as a unit cell.

Figure 22 illustrates a typical clathrate where the guest, benzene, is clathrated in the host, ammonia–nickel cyanide. Since a crystalline structure cannot be easily deformed, a particular cage retains only molecules or atoms within a definite size and shape range. The lower limit of size is determined by the openings in the cage walls through which the guest components could escape. Two series of clathrates may have a common host component, but different cage structures, and contain physically different guest components. Moreover, the unit cells of some clathrates contain cages of two different sizes. A single clathrate of this type may have two different guest components.

The two components of a clathrate do not react chemically with each other, and a chemical equation implying bonds and chemical stoichiometry is inapplicable. Instead, a simple clathrate may be designated by a maximum-composition formula of $(nC)(mM)$, where C and M are the host and guest components, respectively; n is the number of C molecules per unit cage cell; and m is the maximum number of M molecules that can be accommodated in a single cage. The formula $(n/m)(CM)$ may also be used (139).

A special case of clathrate is a self-clathrate, built by incorporation of two identical but independent frameworks of the same compound. The polymorph VI of ice is built this way, where two independent tetrahedral frameworks interpenetrate but are not interconnected by hydrogen bonds. Each framework fills the void space in the other and, in reality, forms a self-clathrate (65).

Crystal Structures and Examples of Clathrates—Crystal structures of different clathrates have one thing in common; that is, they have enclosed spaces, which may be empty, partially occupied, or totally occupied with one or more kinds of guest molecules. On the other hand, there is no common structural characteristic of the enclosing part. The guest molecules, which have nonbonded distances with the host, have a nonstoichiometric relationship with each other. These guest molecules also have some interaction with their cage but cannot be firmly trapped in a cage that is not itself firmly constructed. This implies that the component parts of a host cage should be strongly bound together. The binding between the host atoms may be classified as covalent bonds, ionic bonds, metallic bonds, hydrogen bonds, or van der

[2] The general subject of inclusion compounds will be reviewed later (137).

Waals bonds. In any cage structure, more than one of these types may be involved.

The major classes of clathrates are hydroquinone clathrates, water clathrates[3], phenol clathrates, and clathrates of Dianin's compound. The only official monograph of a pharmaceutical clathrate is that of sodium warfarin clathrate in USP XVIII (140). This clathrate consists principally of sodium warfarin, isopropyl alcohol, and water, where the molecular proportions vary between 8:4:0 and 8:2:2. The expenditure of more time and effort will surely result in the discovery of more pharmaceutical clathrates as has happened with pharmaceutical polymorphs and solvates during the last 15 years.

Preparation of Clathrates—Preparation of most clathrates can be done easily under ordinary laboratory conditions. Most clathrates are generally prepared by recrystallization from solution, where the composition of the clathrate depends on the availability of the guest component at the site of crystal growth. Whenever a host is soluble in the guest, the preparation of the clathrate proceeds without difficulty, and a maximum clathrate composition is realized. Some problems may arise where a common solvent for both host and guest must be used. For example, when the guest concentration is low, slow crystallization with stirring is advised to ensure adequate concentration of the guest at the site of crystallization. Another problem is the choice of the common solvent, which should be inert as far as clathrate formation with the host.

In the case of clathrates formed with gases, high pressure is used to increase the concentration of the second component; at ordinary pressures, the solubility of the gas in the crystallization medium is not sufficient. This method was used to prepare hydroquinone clathrates of the inert gases argon (141), krypton (142), and xenon (143). A most unusual way of obtaining clathrates of substances that might not enclathrate has been suggested (138). This method involves the enclathration of molecules which will decompose within the host cage. Two factors must be satisfied: (*a*) the enclathrated compound must be capable of decomposition by the action of heat, UV light, or X-rays; and (*b*) the cage component must be stable under the decomposition conditions. For more details of the formation of clathrates, the reader is directed to the excellent review by Hagan (144).

Characterization of Clathrates—*Methods Used to Identify Clathrates*—X-Ray diffraction studies on a single crystal is the only sure way to construct the structure of clathrates totally. This is true for any crystalline modification of pharmaceuticals, be it polymorphs, solvates, or clathrates. Rayner and Powell (145, 146), using single crystals, found the crystal structure of the clathrate of benzene with an ammonia–nickel cyanide complex (Fig. 22) and also the crystal structure of hydrated nickel cyanide ammoniate.

X-Ray powder diffraction, although not as powerful a tool as the single-crystal X-ray studies, is a very potent tool for identification purposes. McAdie (147), in his work on urea–*n*-paraffin inclusion compounds, used this technique to differentiate urea from the urea–*n*-paraffin inclusion compound. This technique was not sensitive enough to differentiate the different chain-length paraffins. This finding might mean that once the host cage of an inclusion compound is formed, be it channel, cage, or layer type, X-ray powder diffraction does not vary with the change of the guest molecule. Another interesting observation was made by Mesley (148), who found that the X-ray powder diffraction of clathrates formed by chloroform with hydrocortisone, dexamethasone acetate, and prednisone did not change with various chloroform contents and only reverted to the pure steroid diffraction pattern when all chloroform was removed by heating.

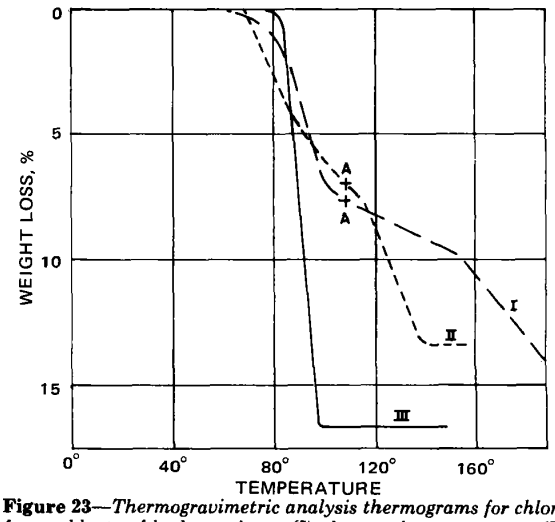


Figure 23—*Thermogravimetric analysis thermograms for chloroform adducts of hydrocortisone (I), dexamethasone acetate (II), and prednisolone (III). (Reproduced, with permission, from Ref. 148.)*

Due to the change of habits of different clathrates, microscopy, both plain and polarized, can be used to identify clathrates. Powell (141), in his work with clathrates of hydroxyquinone with argon, differentiated the large rhombohedra crystals of the clathrate from needle-shaped crystals of α-hydroxyquinone.

Using thermogravimetric analysis, Mesley (148), showed the thermogravimetric curves of hydrocortisone, dexamethasone acetate, and prednisone clathrates with chloroform (Fig. 23). According to this investigator, the chloroform adducts of hydrocortisone and dexamethasone acetate differed from that of prednisone in two respects. The thermogravimetric curves showed two distinct stages of weight loss compared with the rapid and complete decomposition of the prednisone compound. If the thermal decomposition of hydrocortisone and dexamethasone acetate is interrupted at points A in Fig. 23, the materials are still in the crystal form of the clathrate but contain relatively little chloroform. Thus, the initial weight loss is due to removal of chloroform from the lattice, while the second stage corresponds to the

[3] This group should not be confused with crystalline hydrates, as discussed in the solvate section. Here the water is the host of a clathrate host–guest system; in hydrates, water is the solvent of solvation.

breaking up of the lattice. In the case of hydrocortisone, this is followed immediately by steroid decomposition (148).

McAdie (147), working with urea–*n*-paraffin inclusion compounds, used differential thermal analysis. Although these were not clathrates as such, he differentiated the different chain lengths of the *n*-paraffins in the inclusion compounds. This result was just the opposite of that from X-ray powder diffraction, where he was unable to differentiate the inclusion compounds.

One common method used to identify clathrates has been IR spectrophotometry. Using IR, Mesley (148) was able to distinguish the chloroform both in the lattice and on the surface of hydrocortisone and dexamethasone acetate clathrates. Staveley (149) reviewed all of the different spectroscopic studies used in identifying clathrates including IR spectra, nuclear quadrupole resonance spectra, paramagnetic resonance studies, and NMR studies.

Methods Used to Identify the Guest Molecule—Most methods involve breaking the cage structure of the host to liberate the guest molecule for identification purposes.

Lowther and Williams (121) used a grating IR spectrophotometer with a conventional gas cell for identification of the inclusion compound in several clathrated pharmaceuticals found in the British Pharmacopoeia of 1968. This was done by liberating the guest by heat, trapping in a gas cell, and recording the absorption spectrum of the vapor. They were also able to calculate the percentage of solvent found in the clathrate.

Lowther and Williams (121) also used the GC method described in the British Pharmacopoeia of 1968 to identify the guest solvent in several pharmaceuticals.

Using mass spectrometry, Mandelcorn *et al.* (150), while working with the sulfur hexafluoride clathrate of Dianin's compound, calculated the amount of sulfur hexafluoride by melting the clathrate and determining the amount of gas released with a mass spectrometer.

Baker *et al.* (151) were able to use elemental analysis of the adduct when it contained a high proportion of nitrogen, sulfur, and halogen.

Titration can also be used to identify the guest molecule. If the adduct is an acid, the clathrate is dissolved in a suitable solvent and titrated with a suitable alkali (151).

Methods Used to Analyze Host–Guest Physical–Chemical Relationships—There is no doubt that a total X-ray diffraction analysis using single crystals is the only way to identify the host–guest physical–chemical relationships. This is illustrated by work on the crystal structure of the clathrate of benzene with an ammonia–nickel cyanide complex (145) and by work on the chlorine clathrate of water (152).

Other than X-ray diffraction, IR spectrophotometry is also used to check the host–guest physical–chemical relationships. Mandelcorn *et al.* (150), using this technique, were able to find that the interaction between sulfur hexafluoride and its enclosing Dianin's compound cage is very weak.

Properties of Clathrates—Clathrate compounds exhibit the general behavior of molecular compounds. They might have some properties that are those of the host, while others might indicate the presence of the guest. Also, some properties may be seen that are variations resulting from the mutual effect of the host and guest in close proximity.

Molecular Properties—According to Mandelcorn, the host of a clathrate is characterized by one or more permanent dipoles and/or an awkward molecular shape. Molecules not possessing such characteristics are usually arranged, in the crystalline state, as closely packed aggregates; the intermolecular distances are so small that the empty crystalline spaces cannot accommodate molecules larger than those of hydrogen. These molecular properties of the host cause intermolecular interactions and orientations in the crystalline state that lead to the open molecular arrangements essential for a clathrate structure (139). The clathrate structure of Dianin's compound is an example of the effects of both a permanent dipole and an awkward molecular shape (153).

Some weak interactions between a guest molecule and its surrounding cage are similar to those found in physical adsorption. These interactions range from very weak van der Waals intermolecular attractions to highly oriented dipole contacts. The resultant forces contribute to the stability of a clathrate. However, molecules whose interactions with the host are great enough to create new molecular species cannot form clathrates. Hydrogen sulfide interacts very weakly with water; it therefore forms a clathrate with water because of its appropriate molecular size. However, hydrogen chloride reacts chemically with water to form hydronium and chloride ions rather than remain undissociated in a water clathrate structure which accommodates molecules of that size (139).

Thermodynamics—Van der Waals (154) gave a statistical mechanical description of clathrate compounds of nonpolar gases. He first derived the general form of the partition function for a clathrate crystal, while leaving the potential field within a cavity unspecified, and then discussed the thermodynamic consequences of the general formulation. He calculated the potential field within the cavities using the Lennard-Jones and Devonshire method. This method permits the expression of thermodynamic properties of clathrates in terms of a few simple parameters.

Thermal Decomposition—According to Mandelcorn (139), the thermal decomposition of a clathrate into its host and guest at temperatures below its melting point may involve one or more of the following:

1. Sublimation of the host.
2. Change of the clathrate structure to the nonclathrate form of the host.
3. Diffusion of guest molecules through the lattice structure of the clathrate.
4. Desorption of the guest from the surface of the solid clathrate.

All of these processes, except Process 2, are characterized by kinetic expressions that include the geom-

etry of the decomposing clathrate particles. The activation energies of Processes 1 and 2 are determined by the crystalline lattice energies of the clathrate. The activation energy of Process 3 is determined by the lattice energy and guest molecule–cage interaction energy, and the activation energy of Process 4 is determined by the forces of attraction of the guest molecule to the surface of the solid clathrate. A kinetic interpretation of decomposition data can be extremely difficult when more than one of these processes is occurring (139).

Pharmaceutical Application of Clathrates—Although most applications of clathrates are not useful pharmaceutically, a brief review will be given of uses with potential application.

Purification—Evans *et al.* (155) purified benzene of one of its usual contaminants (thiophene) by clathrate formation. Although both thiophene and benzene form clathrates with monoamminenickel cyanide, benzene is more firmly held in the cage structure, so it is preferentially clathrated. The solid clathrate formed between benzene and monoamminenickel cyanide is separated from the solution by filtration. On dry distillation of the clathrate, the thiophene-free benzene is readily recovered.

Separation of Rare Gases—Rare gases were separated by selective clathration (156). Argon is separated from neon by adjusting the pressure conditions under which the hydroquinone–argon clathrate is formed; under the same pressure condition, neon does not form a clathrate with hydroquinone.

Separation of Optical Isomers—Another inclusion complexing substance that will separate optical isomers is tri-*o*-thymotide. This molecule is nonplanar and lacks both a mirror plane and a center of symmetry. It forms two stereochemical configurations, which resemble a three-bladed propeller and are optically active and interconvertible by heating. Slow crystallization provides the chance formation of either the *l*- or *d*-form. These forms have inclusion cavities that are mirror images. Thus, crystallization of trithymotide from a reactive solvent that is also a racemic mixture results in the preferential inclusion of either the *l*- or *d*-form of the solvent. This agent has been used to achieve the optical resolution of *sec*-butyl bromide (157).

Storage of Inert Gases—Hydroquinone–inert gas clathrates are granular materials whose gaseous constituents may be about 10% of the total weight. When the solid volume is taken into account, this gas concentration would exert a pressure of 90 atm in a similar free volume space. The gas can be released by heating or dissolving the clathrate. Therefore, these clathrates may be used for convenient storage of inert gases or to introduce such gases into fairly inaccessible locations. A predetermined amount of clathrate may be sealed into a container. Then, when heat is applied, the container is filled with a desired amount of gas (139, 141). This concept of storage of gases on a commercial basis was patented by Mandelcorn *et al.* (158).

Handling of Dangerous Materials—Cross *et al.* (159) found that dimethylmercury, which is a volatile and extremely toxic compound, can form a stable clathrate with 4-*p*-hydroxyphenyl-2,2,4-trimethylthiochroman as a host. This clathrate is very convenient for preparing solutions of known concentration of dimethylmercury; the preweighed quantity of the adduct is just dissolved in the appropriate solvent. Controlled release of guests that are difficult or hazardous to handle in the free state can be of considerable value in, for example, organic synthesis.

Mode of Action of Anesthetics—Pauling (160, 161), in his hydrate–microcrystal theory of anesthesia, proposed that nonhydrogen-bonding anesthetics work primarily due to the clathrate formation of the molecules of the anesthetic agent with the water contained in the neurons and around the neural network, both in the cells of the neurons and the synaptic regions between the neurons. These water molecules may trap some electrically charged side chains of neuronal proteins and interfere with the movement of ions in the synapses and neurons in such a way as to increase the impedance to the reverberating electric oscillations, thus reducing electrical activity to the point at which consciousness is lost (162).

CONCLUSIONS

Different techniques of identifying habits and characterization of amorphous or crystalline solids, along with their pharmaceutical application, have been discussed. Special emphasis has been given to differentiate polymorphs from molecular adducts, which can occur both stoichiometrically as solvates or nonstoichiometrically as clathrates. The rewards to formulators who characterize the habits and crystalline modification of active drugs and study their cause–effect relationships as part of preformulation studies include: *(a)* fewer processing and shelflife problems, along with rapid solution to problems that do arise; *(b)* the best bioavailable formulations; and *(c)* controlled production of the most efficacious dosage forms.

With all of these examples of the effects of habits, polymorphs, solvates, and clathrates on optimizing pharmaceutical formulations, this reviewer's hope is that crystal chemistry will become a routine part of every pharmaceutical company's preformulation program.

REFERENCES

(1) J. Haleblian and W. McCrone, *J. Pharm. Sci.*, **58**, 911(1969).

(2) S. Rosenstein and P. Lamy, *Amer. J. Hosp. Pharm.*, **26**, 598(1969).

(3) M. Hoeke, *Pharm. Weekbl.*, **104**, 935(1969).

(4) L. da Silva Carvalho, *Rev. Port. Pharm.*, **21**(1), 8(1971).

(5) E. Saias, *Ann. Pharm. Fr.*, **29**, 263(1971).

(6) D. Simmons, R. Ranz, P. Picotte, and S. Szabolcs, *Can. J. Pharm. Sci.*, **5**, 49(1970).

(7) H. Waldmann, *ibid.*, **6**, 76(1971).

(8) D. Simmons, *ibid.*, **6**, 77(1971).

(9) N. H. Hartshorne and A. Stuart, "Practical Optical Crystallography," American Elsevier, New York, N.Y., 1964, pp. 1–46.

(10) J. Grzymek, *Przemysl Chem.*, **21**, 279(1937); through *Chem. Abstr.*, **32**, 2802(1938).

(11) R. S. Tipson, in "Separation and Purification Part I," vol. III, A. Weissberger, Ed., Interscience, New York, N.Y., 1956, p. 433.

(12) A. Wells, *Phil. Mag.*, **37**, 184(1946).
(13) J. B. L. de Rome de L'Isle, "Cristallographie," vol. 1, 2nd ed., L'Imprimerie de Monsieur, Paris, France, 1783, p. 379.
(14) A. F. Compte de Fourcroy and L. N. Vauquelin, *Ann. Chim. Phys.*, **32**, 130(1799).
(15) J. Whetstone, *Trans. Faraday Soc.*, **51**, 973(1955).
(16) J. Whetstone, *J. Chem. Soc.*, **1956**, 4841.
(17) *Ibid.*, **1957**, 4284.
(18) *Ibid.*, **1957**, 4289.
(19) J. Biles, *J. Pharm. Sci.*, **51**, 601(1962).
(20) E. E. Jelley, in "Physical Methods," part II, vol. I, A. Weissberger, Ed., Interscience, New York, N.Y., 1959, p. 1395.
(21) E. E. Wahlstrom, "Optical Crystallography," 3rd ed., Wiley, New York, N.Y., 1962, pp. 13, 14.
(22) J. Shell, *J. Pharm. Sci.*, **52**, 100(1963).
(23) J. Piccolo and R. Tawashi, *ibid.*, **60**, 59(1971).
(24) J. Randall and H. Rooksby, *Glass*, **8**, 234(1931).
(25) J. Randall and H. Rooksby, *J. Soc. Glass Technol.*, **17**, 287(1933).
(26) J. Randall, H. Rooksby, and B. Cooper, *Nature*, **125**, 458(1930).
(27) J. Randall, H. Rooksby, and B. Cooper, *J. Soc. Glass Technol.*, **14**, 219(1930).
(28) J. Randall, H. Rooksby, and B. Cooper, *Z. Kristallogr.*, **75**, 196(1930).
(29) J. Haleblian, R. Koda, and J. Biles, *J. Pharm. Sci.*, **60**, 1485(1971).
(30) T. Kimura and S. Hashimoto, Japanese pat. 60-5798 (1960).
(31) C. Tamura and H. Kuwano, *Yakugaku Zasshi*, **81**, 755(1961).
(32) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 333–335.
(33) J. Mullins and T. Macek, *J. Amer. Pharm. Ass., Sci. Ed.*, **49**, 245(1960).
(34) M. Kuhnert-Brandstätter, "Thermomicroscopy in the Analysis of Pharmaceuticals," Pergamon Press, Oxford, England, 1971.
(35) M. Buerger, *Trans. Amer. Crystallogr. Ass.*, **7**, 1(1971).
(36) M. Perrin and P. Michel, *Acta Crystallogr.*, **B29**, 253(1973).
(37) *Ibid.*, **B29**, 258(1973).
(38) R. Kahn, R. Fourme, D. Andre, and M. Renaud, *Acta Crystallogr.*, **B29**, 131(1973).
(39) B. Busetta, C. Courseille, and M. Hospital, *ibid.*, **B29**, 298(1973).
(40) M. Kuhnert-Brandstätter and S. Wunsch, *Mikrochim. Acta*, **1969**, 1297.
(41) O. Karacsonyi, K. Nikolics, and G. Bidlo, *Acta Pharm. Hung.*, **42**, 203(1972).
(42) *Ibid.*, **42**, 241(1972).
(43) S. Yang and J. K. Guillory, *J. Pharm. Sci.*, **61**, 26(1972).
(44) H. Lin and J. K. Guillory, *ibid.*, **59**, 972(1970).
(45) M. Moustafa and J. Carless, *J. Pharm. Pharmacol.*, **21**, 359(1969).
(46) L. Shenouda, *J. Pharm. Sci.*, **59**, 785(1970).
(47) R. Mesley, *J. Pharm. Pharmacol.*, **23**, 687(1971).
(48) M. Moustafa, A. Ebian, S. Khalil, and M. Motawi, *ibid.*, **23**, 868(1971).
(49) S. Khalil, M. Moustafa, A. Ebian, and M. Motawi, *J. Pharm. Sci.*, **61**, 1615(1972).
(50) L. Borka, *Acta Pharm. Suecica*, **7**, 1(1970).
(51) *Ibid.*, **8**, 365(1971).
(52) A. Aguiar, *J. Pharm. Sci.*, **58**, 963(1969).
(53) A. Aguiar and J. Zelmer, *ibid.*, **58**, 983(1969).
(54) S. Banerjee, A. Bandyopadhyay, R. Bhattacharjee, A. Mukherjee, and A. Halder, *ibid.*, **60**, 153(1971).
(55) M. Miyamoto, T. Kiyotaki, N. Kisoh, T. Mitsunaga, and T. Maeda, *Chem. Pharm. Bull.*, **21**, 1857(1973).
(56) R. Tawashi, *Science*, **160**, 76(1968).
(57) R. Tawashi, *J. Pharm. Pharmacol.*, **21**, 701(1969).
(58) M. Summers, J. Carless, and R. Enever, *ibid.*, **22**, 615(1970).
(59) L. Borka, *Acta Pharm. Suecica*, **9**, 115(1972).
(60) A. Mitchell, B. Milaire, D. Saville, and R. Griffiths, *J. Pharm. Pharmacol.*, **23**, 534(1971).
(61) F. Jamali and A. Mitchell, *Acta Pharm. Suecica*, **10**, 343(1973).
(62) R. Pfeiffer, *J. Pharm. Pharmacol.*, **23**, 75(1971).
(63) G. Schwartzman, *ibid.*, **24**, 169(1972).
(64) B. Mulley, R. Rye, and P. Shaw, *ibid.*, **23**, 904(1971).
(65) B. Kamb, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Eds., Freeman, San Francisco, Calif., 1968, p. 507.
(66) G. Vezzoli, F. Dachille, and R. Roy, *Science*, **166**, 218(1969).
(67) L. Silver and R. Rudman, *J. Phys. Chem.*, **74**, 3134(1970).
(68) M. Vogel, E. Barrall, II, and C. Mignosa, *Mol. Cryst.*, **15**, 49(1971).
(69) F. Fontani, A. Marai, and F. Sbarbaro, *Farmaco, Ed. Sci.*, **26**, 329(1971).
(70) T. Nielson and L. Borka, *Acta Pharm. Suecica*, **9**, 503(1972).
(71) N. Stanley-Wood and G. Riley, *Pharm. Acta Helv.*, **47**, 58(1972).
(72) J. Clements and S. Poli, *Can. J. Pharm. Sci.*, **8**, 88(1973).
(73) A. Shah and E. Strzelinski, presented at APhA Academy of Pharmaceutical Sciences, Washington, D.C., meeting, Apr. 12–17, 1970.
(74) L. Ravin, E. Shamie, and E. Rattie, *J. Pharm. Sci.*, **59**, 1290(1970).
(75) S. Lin, *ibid.*, **61**, 1423(1972).
(76) M. Kuhnert-Brandstätter and A. Burger, *Pharm. Ind.*, **34**, 187(1972).
(77) *Ibid.*, **34**, 353(1972).
(78) *Ibid.*, **34**, 496(1972).
(79) J. Clements and D. Stanski, *Can. J. Pharm. Sci.*, **6**, 9(1971).
(80) M. Munshi and A. Simonelli, presented at the APhA Academy of Pharmaceutical Sciences, San Diego meeting, Nov. 11–14, 1973.
(81) H. Nogami, T. Nagai, E. Fukuoka, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, **17**, 23(1969).
(82) M. Moustafa, S. Khalil, A. Ebian, and M. Motawi, *J. Pharm. Pharmacol.*, **24**, 921(1972).
(83) M. Kanke and K. Sekiguchi, *Chem. Pharm. Bull.*, **21**, 878(1973).
(84) K. Sekiguchi, M. Kanke, Y. Tsuda, K. Ishida, and Y. Tsuda, *ibid.*, **21**, 1592(1973).
(85) D. Simmons, R. Ranz, N. Gyanchandani, and D. Picotte, *Can. J. Pharm. Sci.*, **7**, 121(1972).
(86) J. Pearson and G. Varney, *J. Pharm. Pharmacol.*, **21**, 605(1969).
(87) A. Ebian, M. Moustafa, S. Khalil, and M. Motawi, *ibid.*, **25**, 13(1973).
(88) M. Munshi and A. Simonelli, presented at APhA Academy of Pharmaceutical Sciences, Washington, D.C., meeting, Apr. 12–17, 1970.
(89) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 902.
(90) "Steroid Products for Industry and Research," Searle Chemicals, Chicago, Ill., 1972.
(91) M. Kuhnert-Brandstätter and P. Gasser, *Microchem. J.*, **16**, 419(1971).
(92) *Ibid.*, **16**, 590(1971).
(93) E. Shefter and T. Higuchi, *J. Pharm. Sci.*, **52**, 781(1963).
(94) M. Kuhnert-Brandstätter and H. Grimm, *Mikrochim. Acta*, **1968**, 115.
(95) *Ibid.*, **1968**, 127.
(96) *Ibid.*, **1968**, 577.
(97) J. Shell, *Anal. Chem.*, **27**, 1665(1955).
(98) J. Carless, M. Moustafa, and H. Rapson, *J. Pharm. Pharmacol., Suppl.*, **18**, 190S(1966).
(99) J. Biles, *J. Pharm. Sci.*, **52**, 1066(1963).
(100) H. Rose, R. Hinch, and W. McCrone, *Anal. Chem.*, **25**, 993(1953).
(101) P. Olesen and L. Szabo, *Nature*, **183**, 749(1959).
(102) L. Borka, P. Kristiansen, and K. Backe-Hansen, *Acta Pharm. Suecica*, **9**, 573(1972).
(103) J. Poole, G. Owen, J. Silverio, J. Freyhof, and S. Rosenman, *Curr. Ther. Res.*, **10**, 292(1968).
(104) K. Austin, A. Marshall, and H. Smith, *Nature*, **208**, 999(1965).
(105) J. Chapman, J. Page, A. Parker, D. Rogers, C. Sharp, and

S. Staniforth, *J. Pharm. Pharmacol.*, **20**, 418(1968).
(106) I. Himuro, Y. Tsuda, K. Sekiguchi, I. Horikoshi, and M. Kanke, *Chem. Pharm. Bull.*, **19**, 1034(1971).
(107) K. Sekiguchi, I. Horikoshi, and I. Himuro, *ibid.*, **16**, 2495(1968).
(108) H. Lin, *Diss. Abstr. Int.*, **B**, 4703B(1972).
(109) L. Brancone and H. Ferrari, *Microchem. J.*, **10**, 370(1966).
(110) J. Trivedi, J. Shell, and J. Biles, *J. Amer. Pharm. Ass., Sci. Ed.*, **48**, 583(1959).
(111) H. Nogami, T. Nagai, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, **17**, 499(1969).
(112) H. Caldwell, *J. Pharm. Sci.*, **62**, 334(1973).
(113) A. Holden and P. Singer, "Crystals and Crystal Growing," Doubleday, Garden City, N.Y., 1960, pp. 67–82.
(114) F. Daniels and R. Alberty, "Physical Chemistry," 2nd ed. Wiley, New York, N.Y., 1961, p. 256.
(115) J. Cox, G. Woodard, and W. McCrone, *J. Pharm. Sci.*, **60**, 1458(1971).
(116) J. Hirtz, D. Bouthors, A. Gerardin, and P. Vassort, *Ann. Pharm. Fr.*, **26**, 749(1968).
(117) B. Busetta, C. Courseille, and M. Hospital, *Acta Crystallogr.*, **B29**, 2456(1973).
(118) U. Thewalt and C. Bugg, *ibid.*, **B29**, 615(1973).
(119) L. Niinisto and L. Larsson, *ibid.*, **B29**, 623(1973).
(120) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 947.
(121) T. Lowther and W. Williams, *Analyst*, **96**, 598(1971).
(122) G. Pake, *J. Chem. Phys.*, **16**, 327(1948).
(123) J. McGrath and A. Silvidi, *ibid.*, **39**, 3017(1963).
(124) B. Pedersen, *ibid.*, **41**, 122(1964).
(125) J. Haleblian, R. Koda, and J. Biles, *J. Pharm. Sci.*, **60**, 1488(1971).
(126) S. Hill, H. Seager, and C. Taskis, *J. Pharm. Pharmacol., Suppl.*, **24**, 152P(1972).
(127) B. Ballard and J. Biles, *Steroids*, **4**, 273(1964).
(128) B. Ballard and E. Nelson, *J. Pharmacol. Exp. Ther.*, **135**, 120(1962).
(129) K. Sekiguchi, K. Ito, E. Owada, and K. Ueno, *Chem. Pharm. Bull.*, **12**, 1192(1964).
(130) K. Sekiguchi, *J. Soc. Mater. Sci.*, **21**, 502(1972).
(131) S. Brunauer, P. Emmett, and E. Teller, *J. Amer. Chem. Soc.*, **60**, 309(1938).
(132) Yamanouchi Pharmaceutical Co., Japanese pat. 7,222,716 (June 24, 1972).
(133) G. Brenner, F. Roberts, A. Hoinowski, J. Budavari, B. Powell, D. Hinkley, and E. Schoenewaldt, *Angew. Chem. Int. Ed.*, **8**, 975(1969).
(134) W. Schlenk, Jr., *Ann. Chem.*, **565**, 204(1949).
(135) W. Schlenk, Jr., *Sven. Kem. Tidskr.*, **67**, 435(1955).
(136) M. M. Hagan, "Clathrate Inclusion Compounds," Reinhold, New York, N.Y., 1962, p. 6.
(137) S. Frank, *J. Pharm. Sci.*, in press.
(138) H. Powell, *J. Chem. Soc.*, **1948**, 61.
(139) L. Mandelcorn, *Chem. Rev.*, **59**, 827(1959).
(140) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 675.
(141) H. Powell, *J. Chem. Soc.*, **1950**, 298.
(142) *Ibid.*, **1950**, 300.
(143) *Ibid.*, **1950**, 468.
(144) M. M. Hagan, "Clathrate Inclusion Compounds," Reinhold, New York, N.Y., 1962, pp. 117–142.
(145) J. Rayner and H. Powell, *J. Chem. Soc.*, **1952**, 319.
(146) *Ibid.*, **1952**, 3412.
(147) H. McAdie, *Can. J. Chem.*, **40**, 2195(1962).
(148) R. J. Mesley, *Chem. Ind. (London)*, **43**, 1594(1965).
(149) L. A. K. Staveley, in "Non-stoichiometric Compounds," L. Mandelcorn, Ed., Academic, New York, N.Y., 1964, pp. 620–626.
(150) L. Mandelcorn, N. Goldberg, and R. Hoff, *J. Amer. Chem. Soc.*, **82**, 3297(1960).
(151) W. Baker, A. Floyd, J. McOmie, G. Pope, A. Weaving, and J. Wild, *J. Chem. Soc.*, **1956**, 2010.
(152) L. Pauling and R. Marsh, *Proc. Nat. Acad. Sci. USA*, **38**, 112(1952).
(153) H. Powell and B. Wetters, *Chem. Ind. (London)*, **1955**, 256.
(154) J. H. Van der Waals, *Trans. Faraday Soc.*, **52**, 184(1956).
(155) R. Evans, O. Ormrod, B. Goalby, and L. Staveley, *J. Chem. Soc.*, **1950**, 3346.
(156) British Oxygen Co. Ltd. and H. Powell, British pat. 678,312 (Sept. 3, 1952); through *Chem. Abstr.*, **47**, 3532a(1953).
(157) H. M. Powell, *Nature*, **170**, 155(1952).
(158) L. Mandelcorn, R. Auxier, and C. Lewis, U.S. pat. 2,949,424 (1960); through *Chem. Abstr.*, **55**, 11364a(1961).
(159) R. Cross, J. McKendrick, and D. Macnicol, *Nature*, **245**, 146(1973).
(160) L. Pauling, *Science*, **134**, 15(1961).
(161) L. Pauling, *Anesth. Analg.*, **43**, 1(1964).
(162) J. Catchpool, in "Structural Chemistry and Molecular Biology," A. Rich and N. Davidson, Eds., Freeman, San Francisco, Calif., 1968, p. 353.

ACKNOWLEDGMENTS AND ADDRESSES

Received from *Syntex Research, Palo Alto, CA 94304*

The author acknowledges the following publishers and/or authors for the permission to reproduce various figures used in this review article: Edward Arnold Ltd., London, England; The Pharmaceutical Society of Great Britain; Dr. R. Tawashi; John Wiley and Sons Inc., New York, N.Y.; Masson & Cie., Paris, France; Pharmaceutical Society of Japan, Tokyo, Japan; Nature, Washington, D.C.; Dr. H. Powell; and Dr. R. Mesley.