

during grinding at 35°C, the δ -form was not changed, while the α -form was transformed to the δ -form by way of the ζ -form. The β -form was apparently transformed directly to the δ -form. Once obtained by grinding, the ϵ -form was transformed to the δ -form under 0% relative humidity at various storage temperatures after an induction time of a few hours.

A common occurrence during grinding is the formation of an amorphous, noncrystalline phase. For instance, the grinding of cephalixin has been found to decrease the degree of crystallinity [13] as well as a wide range of physical properties that depend on the crystalline content of the material [14]. When the material becomes less crystalline, its stability decreases, and the hydrate forms become easier to dehydrate. Similar conclusions were reached with respect to the strength of the crystal lattice when the effect of grinding on the stability of cefixime trihydrate was studied [15]. The degree of interaction between the waters of hydration and the cefixime molecules was weakened by grinding, negatively affecting the solid-state stability.

Four modifications of cimetidine were prepared as phase-pure materials, and each was found to be stable during dry storage [16]. The milling process enabled the conversions of Forms B and C to Form A, while Form A transformed into Form D only upon nucleation. In all cases, the particle size reduction step resulted in substantial formation of the amorphous phase. Compression of the various forms into compacts did not yield any evidence for phase conversion.

The pitfalls that can accompany the development of the amorphous form of a drug substance were shown for the capsule formulation of (3*R*,4*S*)-1,4-bis(4-methoxy-phenyl)-3-(3-phenylpropyl)-2-azetidinone [17]. A variety of spectroscopic techniques were used to study the amorphous-to-crystalline phase transition, and it was determined that problems encountered with dissolution rates could be related to the amount of crystalline material generated in the formulation.

One general finding of milling studies conducted on hydrate species is that the grinding process serves to lower the dehydration temperatures of ground materials, facilitating the removal of lattice water and formation of an amorphous product. This behavior has been noted for cephalixin [13,14], cefixime [15], cyclophosphamide [18], 2-[(2-methylimidazol-1-yl)-methyl]-benzo[*f*]thiochromen-1-one [19], and lactitol [20], to name a few representative examples.

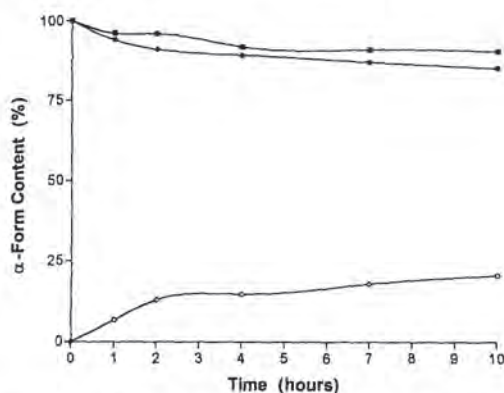


Fig. 3. Changes in α -form content for the α -monohydrate (■), α -anhydrate (●), and β -anhydrate (○) modifications of lactose with grinding time. (The figure is adapted from data presented in Ref. 22.)

Although most studies have been concerned with the state of the drug entity in either bulk or dosage form, milling can also affect the crystalline state of excipients. For example, isomerization was found to take place during the grinding of the α -monohydrate, α -anhydrate, and β -anhydrate forms of lactose [21]. The crystalline materials were all transformed into amorphous phases upon grinding, but these materials quickly re-sorbed water to approach the equilibrium composition ordinarily obtained in aqueous solutions. This behavior has been illustrated in Fig. 3. In a subsequent study [22], these workers determined the degree of crystallinity during various stages of the grinding process and concluded that while the isomerization rate of the α -monohydrate phase depended on the crystallinity, the rates for the two anhydrate phases depended on the content of absorbed water.

IV. EFFECTS DUE TO GRANULATION

When the requirements of a given formulation require a granulation step, this will represent an opportunity for a change in crystal form of

the drug to take place. The problem will be most acute when a wet granulation step is used, since a potential transforming solvent is added to the blend of drug and excipients. The mixture is physically processed in the wet stage until homogeneity is obtained and then dried at a fairly significant temperature. The rationale for including a granulation step is to improve flow and blend homogeneity, permitting the high-speed compression of tablets. The granulation process is used to maintain the distribution of the drug throughout the formulation even during free flow, thus blend homogeneity.

Clearly, one can consider a wet granulation to be equivalent to a suspension of the drug entity in a mixture of solvent and excipients. Since the usual solvent is water, one can encounter a variety of inter-conversions between anhydrides and hydrates, or between hydrates and hydrates, which are mediated by the presence of the solvent. It is equally clear that one should not expect to be able to wet-granulate the metastable phase of a particular compound if that metastable phase is capable of transforming into a more stable form. A discussion of solvent-mediated phase transformations has been given in an earlier chapter and need not be repeated here.

It has been noted that chlorpromazine hydrochloride Form II exhibits severe lamination and capping when compressed, and that wet granulation with ethanol and water significantly improves the tableting characteristics [23]. The reason for this process advantage was shown to entail a phase change of the initial Form II to the more stable Form I during the granulation step. It was concluded that the improvements in tableting and tablet strength that followed the use of wet granulation were due to changes in lattice structure that facilitated interparticulate bonding on compaction.

The effects of granulation solvent on the bulk properties of granules prepared using different polymorphic forms of carbamazepine have been studied [24]. It was found that although the anhydrate phase of the drug transformed to the dihydrate phase in the presence of 50% aqueous ethanol, the phase transformation did not take place when either pure water or pure ethanol was used as the granulating solution. This unusual finding was attributed to the fact that the solubility of the drug in 50% ethanol was 37 times higher than that in pure water. Granules processed using 50% ethanol were harder than those processed with pure solvents and led to the production of superior tablets. This

work demonstrates the extreme need to evaluate the robustness of the granulation step during process development.

The crystal characteristics of a drug substance can undergo a sequence of reactions during wet granulation and subsequent processing. When subjected to a wet granulation step where 22% w/w water was added to dry solids, and the resulting mass dried at 55°C, an amorphous form of (S)-4-[[[1-(4-fluorophenyl)-3-(1-methylethyl)-1*H*-indol-2-yl]-ethynyl]-hydroxyphosphinyl]-3-hydroxybutanoic acid, disodium salt, was obtained [25]. However, this amorphous gradually became crystalline upon exposure to humidity conditions between 33 and 75% RH. Depending on the exact value of the applied humidity, different hydrates of the drug substance could be obtained in the formulation.

V. EFFECTS DUE TO DRYING

Whenever a wet suspension containing a drug substance is dried, the possibility exists that a change in crystal state will take place. It has been amply shown in earlier chapters that anhydrides or amorphates can be produced by the simple desolvation of a solvate species, and such reactions are always possible during any drying step. The production of anhydrate phases by simple drying is one example of a reaction that can accompany the drying process, and this reaction type has been fully discussed in an earlier chapter.

In the simplest type of drying procedure, moist material from the wet granulation step is placed in coated trays and the trays are placed in drying cabinets with a circulating air current and thermostatic heat control. Historically, the tray drying method was the most widely used method, but more recently, fluid-bed drying is now widely used. In drying tablet granulations by fluidization, the material is suspended and agitated in a warm air stream. The chief advantages of the fluid-bed approach are the speed of drying and the degree of control that can be exerted over the process. The wet granulations are not dried to zero moisture; for a variety of reasons one seeks to maintain a residual amount of moisture in the granulation. It is evident from these simple considerations that the combination of solvent and drying conditions provides a suitable environment for the generation of new polymorphs or solvates when such conversion routes are available.

During ordinary manufacturing processes, crystals of the drug substance are dried under vacuum or through the circulation of warm dry air. Evaluation of wet and dried samples of the bulk substance by thermogravimetric analysis and hygroscopicity studies usually yields the necessary insight into the limiting parameters of the drying process. Since drying times can be relatively long and the environmental temperature mild (such as 24 to 48 h at 60°C), partial or total polymorphic conversion is possible. The likelihood that phase conversion will take place becomes increasingly less as the solvent is removed during drying. This area has been thoroughly covered earlier in Chapter 5, so only a few illustrative examples will be quoted at this time.

Shefter et al. used x-ray diffraction analysis to show that overdrying ampicillin trihydrate resulted in an unstable amorphous product, and that the drying of theophylline hydrate yielded a crystalline anhydrous form [26]. Kitamura et al. dehydrated (with vacuum) cefixime trihydrate to obtain a crystalline anhydrous form, which also proved to be unstable [27].

Otsuka et al. studied the effect of humidity and drying on the polymorphic transformation rate at 45°C for six forms of phenobarbital [28]. Figure 4 shows that the monohydrate (Form C) and the hemihydrate (Form E) slowly convert to the anhydrous Form B under high-humidity stress conditions (45°C and 75% RH), while the conversion is very fast at low humidity (45°C and 0% RH). This is reasonable, since the driving force to form anhydrous material is a dry environment. The key point of Figure 4 is the rapid conversion of phenobarbital in less than 12 h at 45°C and 0% RH, which represents very reasonable processing conditions for an industrial drying oven.

The sodium salt of 3-(((3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-dimethylamino)-3-oxo-propyl)thio)propanoic acid represents an unusual case of a hygroscopic drug substance, where the surface-active substance (in both crystalline and lyophilized forms) produced nonflowing, semisolid masses with exposure to increasing relative humidity [29]. The substance was shown to form first an amorphous material and then a mesomorphic phase, as the moisture sorption increased with exposure to relative humidity.

The favorite endpoint of the process chemist is to "dry to constant weight," but this laudable goal can lead to the production of a desol-

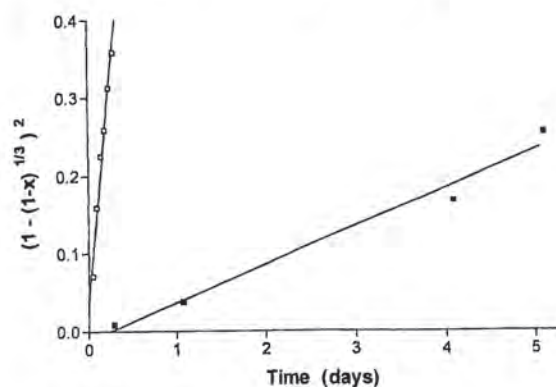


Fig. 4. Jander plots of one polymorphic transformation of phenobarbital. Illustrated are data for the conversion at 45°C of Form C (monohydrate phase) to Form B (anhydrate phase). The 0% relative humidity atmosphere points are given by the open symbols, and the 75% relative humidity atmosphere points are given by the closed symbols. (The figure is adapted from data presented in Ref. 28.)

vated product. Without doubt, the overdrying process has produced many anhydrous lots of bulk drug substance, which unfortunately are eventually found to be unstable.

Since the effects of simple drying have been more fully discussed in detail in Chapter 5 of this book, we will turn to case studies that illustrate how the use of two specialized drying methods can lead to phase interconversions.

A. Changes in Crystalline Form Accompanying the Spray-Drying Process

The spray-drying process first requires the formation of a slurry to be sprayed, which can be a concentrated solution of the agent to be dried or a dispersion of the agent into a suitable nondissolving medium. The dispersion is then atomized into droplets, which are exposed to a heated atmosphere to effect the drying process. Completion of the process yields a dry, freeflowing powder that ordinarily consists of spherical

particles of relatively uniform particle size distribution. For instance, the morphology of spray-dried lactose has been contrasted with that of the monohydrate and anhydrous phases of lactose, with significant differences being reported [30].

One interesting feature of spray-drying is the possibility of controlling the final crystal form through manipulation of the drying conditions. For instance, three different crystalline forms of phenylbutazone were prepared from methylene chloride solution by varying the drying temperature of the atomized droplets between 30 and 120°C [31]. One of the isolated polymorphs could not be obtained by any crystallization procedure and could only be produced using extremely slow solvent evaporation rates.

Using phenobarbitone and hydroflumethiazide as examples, it has been shown that high-energy solids can be produced through the use of spray-drying [32]. Commercially available phenobarbitone is obtained as Form II, but with spray-drying the authors were able to obtain Form III having a large specific surface area. The analogous spray-drying of hydroflumethiazide yielded an amorphous solid. The spray drying of indomethacin produced a viscous glassy phase, which was found to be physically unstable with time [33]. Upon storage, the glassy solid converted into a mixture of crystalline Forms I and II. Representative x-ray powder diffraction patterns of these materials are shown in Fig. 5.

Spray-drying is often used to encapsulate drug substances into excipient matrices, but phase interconversions are still possible in such situations. Sulfamethoxazole Form I was microencapsulated with cellulose acetate phthalate and talc, colloidal silica, or montmorillonite clay by a spray-drying technique, and the drug was found to convert to Form II during the process [34]. Increasing the amount of cellulose acetate phthalate in the formulation led to increased amorphous drug content, but increasing the talc level yielded more polymorphic conversion. In a subsequent study, the authors showed that no phase conversion took place in formulations containing colloidal silica [35].

Frequently, the spray drying process yields amorphous materials, which undergo crystallization upon storage or exposure to suitable environmental conditions. The effect of various additives on the recrystallization of amorphous spray-dried lactose has been studied [36]. The

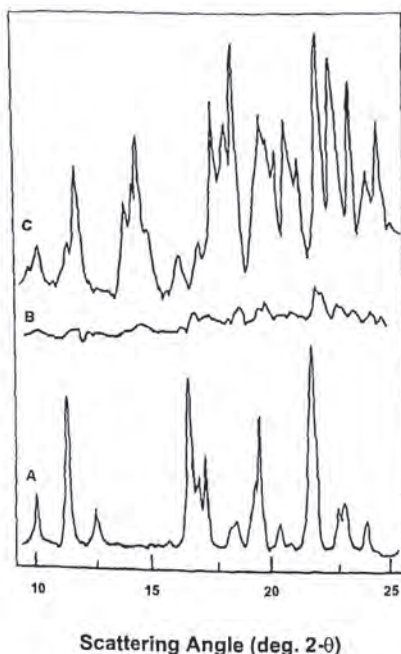


Fig. 5. X-ray powder diffraction patterns for (A) crystalline indomethacin, (B) spray-dried indomethacin, and (C) indomethacin spray-dried with 5% polyvinylpyrrolidone and stored for two months. (The figure is adapted from data presented in Ref. 32.)

presence of magnesium stearate was found to inhibit the recrystallization process, while layers of microcrystalline cellulose in the spray-dried products could result in long lag times prior to recrystallization. It was deduced that the onset of crystallization was critically related to the mobility of water in the formulations.

B. Changes in the Crystalline State of Lyophilized Products

Another drying procedure that can yield changes in drug crystal form is that of freeze-drying, or lyophilization. In this approach, the material

to be dried is prepared as an aqueous solution or suspension and then frozen rapidly and cooled to a temperature below its eutectic point. The frozen formulation is then exposed to vacuum and the ice removed by sublimation. Since lyophilized products are often dried to moisture levels less than 1%, the materials are ordinarily hygroscopic and must be protected from adventitious water to prevent any unwanted phase transformation steps. In usual practice, the product is produced in its amorphous state, which is favorable for its subsequent solubilization at the time of its intended use. Consequently, the study of any possible moisture-induced crystallization is important to the characterization of a lyophilized product.

The effect of storage conditions on the crystalline nature of lyophilized ethacrynate sodium has been reported [37]. Samples of the drug substance were dried to various moisture levels and then stored at either 60°C or 30°C/75% RH. It was found that crystalline drug substance was obtained after short periods of time as long as sufficient levels of water were either present in the initial lyophilized solid or introduced by adsorption of humidity. The crystalline form of the drug was identified as a monohydrate phase.

Cefazolin sodium is capable of existing in a number of crystalline modifications, but the amorphous state is produced by the lyophilization process [38]. The amorphous form retained its nature at relative humidities less than 56% but converted completely to the pentahydrate above 75% RH. Interestingly, the hygroscopicity of the amorphous form was essentially similar to that exhibited by the dehydrated monohydrate or dehydrated α -form. Lamotrigine mesylate was also found to exhibit a moisture-dependent amorphous-to-crystalline transition [39]. The transition was found to be independent of the presence of mannitol, whether the amorphous solid was produced by lyophilization or by spray-drying.

Excipients in a lyophilized product can also undergo amorphous-to-crystalline phase transitions. The transformation of amorphous sucrose to its crystalline phase was studied as a function of applied relative humidity [40]. As illustrated in Fig. 6, the amorphous material gains up to 6% water when exposed to a relative humidity of 33%, but continued storage leads to crystallization of the anhydrous crystalline phase and a consequent expulsion of the initially adsorbed water. It

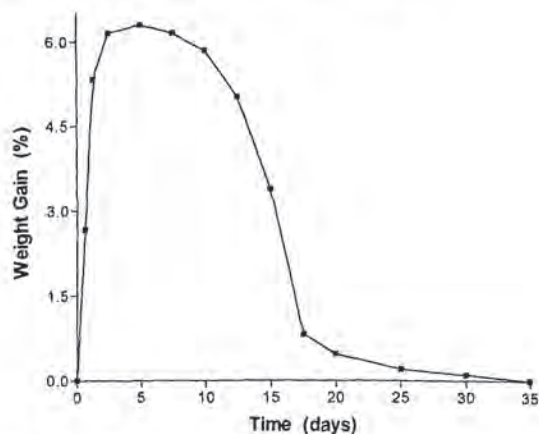


Fig. 6. Moisture uptake curve for amorphous sucrose, obtained at 23°C and a relative humidity of 33%. (The figure is adapted from data presented in Ref. 40.)

was hypothesized that the lag time that accompanied the amorphous-to-crystalline phase transition was required for the random motion of molecules to rotate and translate into the proper orientation to form a nucleus of sufficient size to allow crystallization.

Mannitol is commonly used as a bulking agent in lyophilization, owing to its tendency to freeze-dry into a mixture of crystalline and amorphous solids [41]. However, since mannitol can exist in a number of crystalline modifications, slight variations in the exact details of the lyophilization procedure can lead to the production of differing solids [42]. The effect of surface-active agents on the composition of lyophilized mannitol formulations has been studied, and it was reported that polysorbate 80 affected both the polymorphic state and the degree of crystallinity of freeze-dried mannitol [43]. When lyophilizing pure mannitol, one obtains a mixture consisting of the α , β , and δ forms in various ratios. In the presence of polysorbate 80, however, production of the δ -phase is favored.

VI. EFFECTS DUE TO COMPRESSION

It is often assumed that the crystalline form of a drug entity will remain unchanged during the compression steps associated with the manufacture of tablets. The reason for this line of thought is that under the applied load of tableting, the softer excipients should deform preferentially to the relatively hard crystalline drug substance. As tableting speeds increase towards commercial production, exposure times to stress decrease and one would anticipate even less chance for crystalline conversion. For the production of many substances, this situation is certainly true. There are numerous examples, however, which do not follow the general rule, and where changes in polymorph or solvate form accompany the compression step.

Effects due to compression can be further divided into two categories. One of these concerns the phase transformations that accompany the compaction and consolidation steps of tablet manufacture. The other relates to the effect on tablet properties that arise from the use of different crystal forms in a direct compression tablet formulation. Case studies pertaining to each of these categories will be discussed in turn.

A. Changes in Crystal Form Effected by Compaction

In one study of wide scope, 32 drugs known to exist in different polymorphic states were subjected to a trituration test for possible phase transitions [44]. Out of 11 transforming substances, detailed studies of tableting were conducted on caffeine, sulfabenzamide, and maprotiline hydrochloride. Tablets were produced and sectioned, so that thermal analysis could be used to investigate polymorphic changes at the upper and lower surfaces, middle region, and sides of the compacts. Relationships were examined between the extent of transformation and the compression pressure and energy, as well as the effect of drug particle size. It was deduced that the form with the lowest melting point would have the least degree of intermolecular attractive forces and probably the least yield stress values at a given temperature. This conclusion was supported by the observation that the compression of metastable phases brought about a transformation to the most stable phase. As illustrated for the particular example of caffeine in Fig. 7, the extent of transforma-

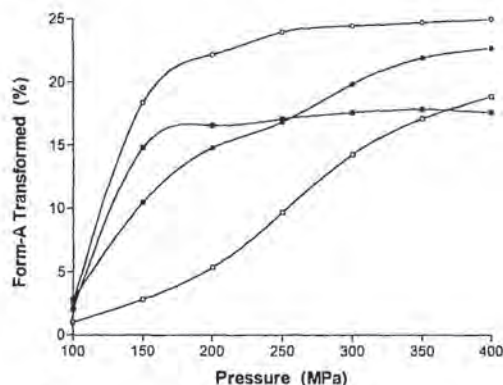


Fig. 7. Percentage of caffeine Form A transformed into Form B as a function of applied pressure. Phase composition data are shown for the upper surface (○), lower surface (□), middle region (■), and side (●) of compressed tablets. (The figure is adapted from data presented in Ref. 44.)

tion was found to depend on the zone of the tablet, the pressure applied, and the particle size of the powder.

Carbamazepine is a drug where irregular plasma levels can be obtained owing to differences in dissolution rates that are associated with the production of differing polymorphs by tablet manufacturing operations. The behavior of the α , β , and dihydrate crystalline forms during compression has been investigated, as well as the possibility of crystalline structural changes accompanying grinding and tableting conditions [45]. Grinding was performed in a ball mill for 15 and 60 minutes, while compression was carried out using an instrumented single punch machine. It was found that although the dihydrate form exhibited the best compressibility characteristics, it was not phase-stable with respect to compression. The α -form exhibited the best stability, but the use of this phase induces sticking in the tablets. It appears that the commercially available β -form represents the best compromise between performance and stability, and it was found to remain stable under normal conditions of fabrication and storage.

The effect of tablet compression mechanical energy on the polymorphic transformation of chlorpropamide has been examined [46]. A single-punch eccentric tableting machine with a load cell and a noncontact displacement transducer was used to measure compression stress, distance, and energy. Both the stable Form A and the metastable Form C were compressed at a stress of 196 MPa at room temperature. The compression cycle was repeated from 1 to 30 times, and the phase composition of deagglomerated tablets was measured using powder x-ray diffraction. It was found that each form could be transformed into the other by the mechanical energy input during tableting, with the composition reaching equilibrium above 100 J/g of compression energy after more than 10 cycles. It was hypothesized that the phase transformation required the conversion of the crystalline forms to an amorphous intermediate, which then transformed into the equilibrium ratio of forms A and C.

The effect of compressional force on the polymorphic transition in piroxicam has been examined for phase-pure materials, where the needle-like α -phase was found to convert to the cubic β -phase during compression [47]. The phase transformation took place only at higher applied forces, such that formation of the α -phase could only be observed after compression to a tablet hardness of 9 kg/cm². Compression of the β -phase yielded no changes in its crystalline form.

The extent of the polymorphic transformation of anhydrous caffeine has been studied as a function of grinding time and compression pressure [48]. It was found that both grinding and compression induced the transformation from the metastable Form I into the stable Form II. The transformation could be observed even after only 1 min of grinding, or by using a tableting compression pressure of about 50 MPa. Quantitative measurements of phase conversion indicated that the degree of transformation was greater near the surface of the tablet than in its interior.

The effect of temperature on polymorphic transformations that take place during compression has been studied. Tableting temperature was found to exert a definite effect on the polymorphic transformation of chlorpropamide during compression, and on the physical properties of the produced tablets [49]. Compression stress, distance, and energy were measured using a noncontact displacement transducer mounted

within a temperature-controlled single punch eccentric tableting machine with two load cells (upper and lower punches). The phase composition of deagglomerated compacts was subsequently measured using powder x-ray diffraction to calculate the polymorphic content. It was learned that the amount of Form C produced at 45°C by the transformation of Form A was about twice that produced at 0°C using the same compression energy. The amount of Form A transformed from Form C by compression at 45°C was almost the same as that at 0°C. This suggested that the compressional effect on Form A depended on the compression temperature, while the effect on Form C was independent of temperature. The crushing strength of tablets produced using Form A was about twice that obtained for tablets produced from Form C, even when they were compressed at the same porosity.

A study of the effect of temperature on the polymorphic transformation and compression of chlorpropamide Forms A and C during tableting has been reported [50]. With the aid of apparatus similar to that previously described [49], these authors used multitableting at room temperature and single tableting at 0–45°C to effect compression. In the first method, the stable Form A or the metastable Form C was loaded into the die and the sample was compressed with a compression stress of 196 MPa. It was found that both forms were mutually transformed, and as evident in Fig. 8, an equilibrium ratio of Forms A and C was attained above roughly 200 J/g of compression energy. In the second method, the amount of Form C transformed from Form A at 45°C was about two times larger than that at 0°C at the same compression energy. The crushing strength of tablets prepared from Form A was about twice that of tablets manufactured using Form C as the source of drug substance.

A further study was conducted to study the effect of compression temperature on the consolidation mechanism of the polymorphs of chlorpropamide [51]. The effect of environmental temperature on the compression mechanism of the A and C forms of chlorpropamide was investigated with an eccentric type tableting machine with two load cells and a noncontact displacement transducer. The temperature of the sample powders was controlled at 0 and 45°C, and these were compressed at almost 230 MPa. The tableting dynamics were evaluated by Cooper and modified Heckel analyses. The results suggest that particle

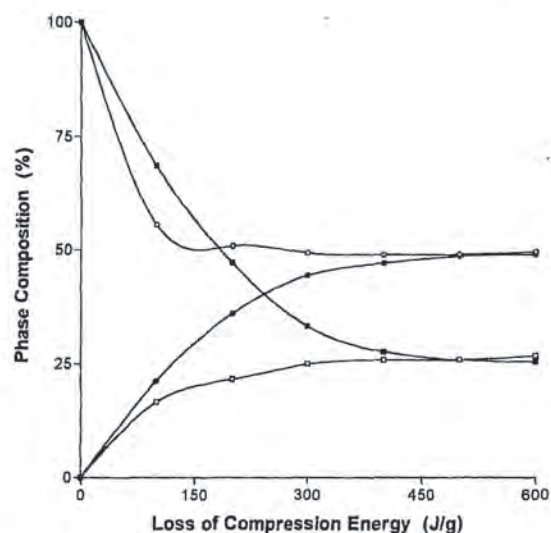


Fig. 8. Relationship between phase composition and compression energy during the compaction of different polymorphs of chlorpropamide. Shown are data for the residual content of Form A during the compression of Form A (○), the amount of Form C formed during the compression of Form A (□), the residual content of Form C during the compression of Form C (■), and the amount of Form A formed during the compression of Form C (●). (The figure is adapted from data presented in Ref. 50.)

brittleness or plasticity was affected by compression at different temperatures. The higher tablet hardness of Form A tablets produced at 45°C was thought to be caused by the increased plasticity of primary particles, whereas the hardness of tablets containing Form C (and also produced at 45°C) was ascribed to the decreased size of the secondary particles. Part of the reasoning behind this conclusion was the observation that Form A melted approximately 8°C lower than did Form C and would therefore be more plastic with respect to deformation.

Sebhatu et al. studied the tableting characteristics of spray-dried lactose [52], which typically contains 15% amorphous material. The spray-dried material is known to absorb moisture when exposed to high

levels of relative humidity, which lowers the glass transition temperature of dry material from 104°C to 37°C (at moisture content 7.17%). It was found that tablets made from spray-dried lactose that had been stored for less than 4 h at 57% RH before compaction showed an increased in postcompaction tablet hardness when stored at 57% RH. This effect was ascribed to crystallization of the amorphous regions brought into close contact by compression. On the other hand, spray-dried lactose stored at 57% RH for more than 6 h prior to compaction showed no change in postcompression hardness for tablets stored at 57% RH because the amorphous regions had completed crystallization prior to compaction. One concludes that any tableted product initially containing a large portion of amorphous material (either active drug or inactive excipient) may be subject to changes in tablet hardness upon storage in high humidity environments.

B. Effects on Tablet Properties Associated with the Use of Different Crystal Forms

The dissolution rate of phenylbutazone from disintegrated tablets has been used to determine whether the drug particles underwent crushing or bonding during compression [53]. By using two polymorphic forms of the drug substance, it was shown that the predominant effect during compression in formulations containing high drug concentrations was dependent upon the original particle size of the active and on its polymorphic state. In formulations having a low drug concentration, the excipients effectively prevented the drug particles from bonding together. Lactose was found to exert a more abrasive action (relative to Avicel) on Form A but had little effect on the more ductile Form B. This effect is illustrated in Fig. 9, where the dissolution rates of tablets produced from the two polymorphs are contrasted. The effect of using differing particle size ranges of input material is particularly evident in the dissolution data of tablets prepared using the finest grade of drug substance. A second aspect of this work concerns the analysis of how the rate of compression during tableting affects the dissolution time. For larger particle sizes of raw material, no difference was observed on passing from 0.26 to 29 of compression time. For the smallest grade of input material, a significant difference was observed. The authors

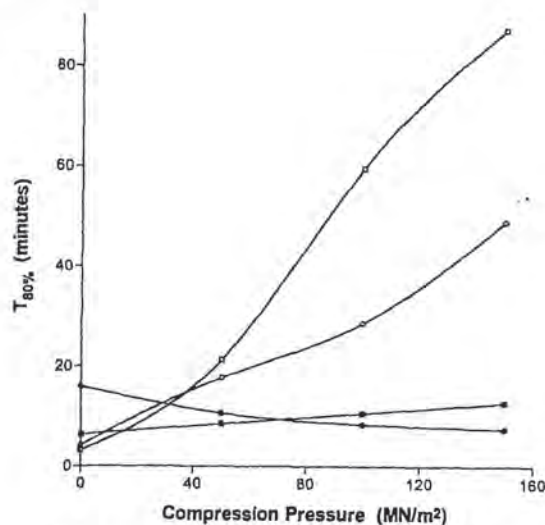


Fig. 9. Change in dissolution rate of disintegrated tablets of phenylbutazone as a function of particle size, compression pressure, and crystal form. Shown are the profiles for tablets prepared from Form A of 6 μm particle size ($\circ$), tablets prepared from Form B of 6 μm particle size ($\square$), tablets prepared from Form A of 137 μm particle size ($\bullet$), and tablets prepared from Form B of 146 μm particle size ($\blacksquare$). (The figure is adapted from data presented in Ref. 53.)

concluded that a decrease in the rate of compression from 29 s to 0.26 s resulted in less bond formation within the tablet matrix and a more rapid dissolution.

The work of Tuladhar et al. [53] is significant because industrial tableting machines operate typically at 200 revolutions per minute. This means 0.3 seconds per revolution (i.e., the exposure time to compression), which is far less than the prolonged exposure on a manual Carver Press (on the order of minutes) or on some of the single-station tablet machines presented earlier (3 revolutions per minute). Prolonged exposure during compression may lead to erroneous conclusions about the significance of polymorphic transformation during tableting. Neverthe-

less, some interesting information can be derived from such studies if they are of proper design.

Others have sought to develop new methods for a better evaluation of the relation between the mechanical strength of solids and the polymorphic form used to produce these [54]. In order to evaluate the role played by polymorphism in the mechanical strength of solid dosage forms, and to minimize the influence of other factors, a melted disc technology was proposed. Tablet-shaped discs of zero porosity were prepared by melting powder and subsequent crystallization in the desired modification. Using phenobarbitone as a model drug, discs consisting of Forms I, II, and III, as well as the amorphous form, were prepared. An evaluation of the mechanical properties of the discs was conducted, primarily through determinations of bending strength. It was found that the amorphous form and Form III yielded the strongest discs and would therefore probably be the most suitable materials for tablet manufacture.

The poor compression of paracetamol was addressed through the preparation of a new orthorhombic form [55]. Owing to its well-known poor compressional properties, commercially available paracetamol materials for direct compression are compounds of paracetamol with gelatin, polyvinylpyrrolidone, starch, or starch derivatives. Since a chemically pure paracetamol that could be used for direct compression would constitute a better compendial article, a new polymorph was produced. The new form was recrystallized from dioxane, and its crystals were found to consist of sliding planes that led to good compressibility. However, the orthorhombic form is metastable with respect to the monoclinic form; phase conversion was observed if the raw material contained greater than 20% of the monoclinic phase. However, the dissolution rates for the two forms were found to be similar, and therefore any questions as to the relative bioavailability of the two forms would probably be meaningless.

Compressive deformation studies were conducted on single crystals of α -lactose, using mechanical strength and acoustic emission analyses to deduce any possible differences in the deformation behavior of α -lactose monohydrate and anhydrous α -lactose [56]. The α -lactose monohydrate crystals were found to exhibit greater mechanical strength

than did the anhydrous α -lactose crystals. The acoustic emission data showed that the fragmentation process of the monohydrate phase was acoustically more active and energetic. Amplitude distribution analysis of the acoustic signals further confirmed that the nature of fragmentation during the deformation of the two types of lactose was different. This was attributed to fundamental differences in the internal crystal structure of the two lactose types. This work showed that mechanical strength and acoustic emission analyses are able to provide significant insights into the fundamental deformation characteristics of different polymorphic or solvate species.

The compressional behavior of four polymorphs of mannitol (the α , β , and δ forms, as well as one unidentified phase) has been studied [57]. It was found that the compressibility of the α -phase is superior, and fortunately this phase is the major form in most commercial products. The particle shape was found to exert an influence upon the compressibility properties; granulated powders show better behavior than native crystalline powders. It was reported that no polymorphic transitions took place under the compression stresses used during the tabletting process.

VII. SUMMARY

The key parameters to consider at the bulk drug substance production stage are prolonged processing times due to batch size, improved product purity, a lengthy final crystallization step to improve purity, improve yield, or increase particle size, drying conditions for the product, and milling to improve homogeneity or reduce particle size. In all these steps, the small energy barriers between crystalline forms are easily overcome, resulting in the wrong polymorphic form, a desolvated product, or an amorphous product.

Once the kilogram bulk has been produced in its preferred polymorphic form, or salt form, or solvate form, it will proceed to the pharmaceuticals department, where it will be mixed with excipients, exposed to processing conditions, and converted to a marketable dosage form. The pharmaceutical scientist must formulate this material into a dosage form that is homogeneous, scalable, stable, and bioavailable. Since

most dosage forms are solid dosage forms, capsules or tablets, this effort will focus primarily on those processing parameters that are operative in the production of solid dosage forms.

Solution dosage forms are ordinarily independent of polymorphic problems, but they require one warning. If there should happen to exist a less soluble form, it will appear upon temperature cycling stability testing. The reason for this solution state warning is that temperature cycling is the most severe challenge to solubility, and if one should generate seed crystals of a less soluble form of a compound during cooling, then equilibrium will rapidly be established. The final stage of this scenario will be precipitation or crystal growth. The classic cases in the industry are the soluble anhydrous material converting to an insoluble hydrate upon stability testing, or moisture leakage into a delivery system.

In solid dosage forms, the first opportunity for a change in crystallinity will be in the blending and milling of drug with excipients to produce a homogeneous blend. The excipients in a formulation can exert a strong influence on polymorphic conversion and may create new pathways that did not exist for the pure drug substance. One should expect polymorphs with similar thermodynamic energies to be prone to substantial conversion during a milling operation.

The key parameters that cause polymorphic conversion or dehydration of hydrates at the formulation stage are milling and granulation wetting. Other factors that can be important are drying, tableting, and drying of the film coating. In addition, one can have migration of water between drug substance, excipients, and capsule shells. All these parameters can become more important during prolonged processing times should the batch size become sufficiently large.

During mechanical treatment, the most likely mechanism for polymorphic interconversion is that of nucleation and growth of a second phase within the original phase. Nucleation proceeds from dislocation sites in a crystal, since their higher free energy ensures that the energy needed for transformation is lower at these sites. In the majority of cases, metastable phases transform to the most stable form. The degree of polymorphic conversion will depend on the relative stability of the phases in question, and on the type and degree of mechanical processing applied. These phase conversions are generally modulated

by the presence of excipients in the formulation, and consequently they must be investigated in the dosage form as well as in the bulk drug substance.

The goal in any industrial pharmaceutical organization is to have the thermodynamically preferred polymorph or solvate present in the first scaled-up batch of drug substance. If this situation is achieved, then all toxicology, pharmacokinetic, and clinical studies will be conducted with the crystalline form that is likely to be the commercial form of the drug substance. This will eliminate expensive retesting should a more stable but previously unknown polymorph appear. Prudent drug development programs will identify the preferred crystalline form early in development, with a polymorph/salt group working in close conjunction with process chemists to make the thermodynamically preferred polymorphic form the initial kilogram-scale batch. They will also identify and define the physicochemical boundaries of that polymorph.

In the end, however, there is no substitute for multidisciplinary studies whose goal is to determine the likelihood of polymorphic interconversions at any time during the handling of the drug substance. Fortunately, the wide variety of available characterization methods makes it possible to detect virtually any problem that could be encountered during the course of drug development. The successful approach to problem solving will require the full collaboration of process, formulation, and analytical scientists and the complete sharing of information. Although problems in development invariably occur at the worst possible moment, the judicious use of information previously gathered from properly designed studies should minimize their impact and lead to ready deductions of methods for their elimination.

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9

Structural Aspects of Molecular Dissymmetry

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I. INTRODUCTION

The molecular chirality associated with an optically active molecule is ordinarily manifested in the crystallography of the compound [1]. Since the historical development of optical activity was greatly aided by systematic studies of the habits of enantiomorphic crystals, the concepts of molecular dissymmetry, crystallography, and chirality are inexorably linked. As with any other solid, these materials can be characterized on the basis of their crystal structures and through an understanding of their melting point phase diagrams.

It has become abundantly clear that the stereoselective actions associated with the enantiomeric constituents of a racemic drug can differ markedly in their pharmacodynamic or pharmacokinetic properties [2-5]. These factors can lead to much concern, especially if a drug containing a potentially resolvable center is marketed as a racemic mixture. This situation is not invariably bad, but it is clear that a racemate should not be administered when a clear-cut advantage exists with the use of a resolved enantiomer [6]. An excellent discussion regarding the possible selection of a resolved enantiomer over a racemate, from both a practical and a regulatory viewpoint, has been provided by De Camp [7].

When the mirror images of a compound are not superimposable, these mirror images are called enantiomers. Numerous techniques exist that permit the physical separation of enantiomers in a mixture [8], but a discussion of these is outside the scope of this work. Individual enantiomeric molecules are completely equivalent in their molecular properties, with the exception of their interaction with circularly polarized light. An equimolar mixture of two enantiomers is termed a racemic mixture. The generally accepted configurational nomenclature for tetrahedral carbon enantiomers was devised by Cahn, Ingold, and Prelog and is based on sequencing rules [9]. Enantiomers are identified

as being either *R* or *S*, depending on the direction (clockwise or counterclockwise) of substituents after they have been arranged according to increasing atomic mass. Compounds containing more than one center of dissymmetry are identified as diastereomers, and in compounds containing n dissymmetric centers the number of diastereomers will equal 2^n .

Although they do not fit the formal definition of a polymorphic solid, the relationship between racemic mixtures and resolved enantiomers presents an intriguing parallel. By virtue of symmetry constraints, a resolved enantiomer must crystallize in a noncentrosymmetric space group. Racemic mixtures are under no analogous constraint, but over 90% of all racemic mixtures (which are merely equimolar mixtures of the enantiomers) are found to crystallize in a centrosymmetric space group [10]. Thus we immediately reach the intriguing situation where differing crystal structures can be obtained for the same chemical compound, depending only on the degree of resolution. Since this type of behavior falls within the confines of this book, we will therefore explore the relation between molecular and crystallographic chirality and examine some of the consequences of these relationships.

II. ENANTIOMER AND RACEMATE CRYSTAL STRUCTURES

A relatively small number of the possible crystallographic lattice symmetries are available for the crystals of separated enantiomers, since these must crystallize in a lattice structure totally devoid of inverse elements of symmetry. Out of the 230 possible space groups belonging to the 32 crystal classes, only 66 space groups within 11 crystal classes are noncentrosymmetric and can accommodate homochiral sets of enantiomers. Racemates are permitted to crystallize in any of the 230 space groups but are not restricted to crystallizing in a centrosymmetric group. It is found that most racemates crystallize into a group that possesses some elements of inverse symmetry. As will be discussed later, racemic mixtures occasionally crystallize in an enantiomorphic lattice system and consequently spontaneously resolve the enantiomers.

A symmetry element is defined as an operation that when per-

formed on an object results in a new orientation of that object that is indistinguishable from and superimposable on the original. There are five main classes of symmetry operations: (a) the identity operation (an operation that places the object back into its original orientation), (b) proper rotation (rotation of an object about an axis by some angle), (c) reflection plane (reflection of each part of an object through a plane bisecting the object), (d) center of inversion (reflection of every part of an object through a point at the center of the object), and (e) improper rotation (a proper rotation combined with either an inversion center or a reflection plane). Every object possesses some element or elements of symmetry, even if this is only the identity operation.

The rigorous group theoretical requirement for the existence of chirality in a crystal or a molecule is that no improper rotation elements be present. This definition is often trivialized to require the absence of either a reflection plane or a center of inversion in an object, but these two operations are actually the two simplest improper rotation symmetry elements. It is important to note that a chiral object need not be totally devoid of symmetry (i.e., asymmetric); it must merely be dissymmetric (containing no improper rotation symmetry elements). The tetrahedral carbon atom bound to four different substituents may be asymmetric, but the reason it represents a site of chirality is by virtue of its dissymmetry.

Jacques et al. have evaluated the compilations published by various authors and reported that 70–90% of homochiral enantiomers crystallize in the $P2_12_12_1$ or $P2_1$ space groups [10]. The most frequently encountered chiral space group, $P2_12_12_1$, is orthorhombic, which on a macroscopic level translates to a system consisting of three orthogonal binary axes. The unit cell commonly consists of four homochiral molecules that are related to each other by three binary screw axes. Space group $P2_1$ belongs to the monoclinic system and is characterized by a plane of symmetry and a twofold axis. The cell generally contains two molecules related by a binary screw axis.

Jacques et al. have also concluded that among the 164 space groups possessing at least one element of inverse symmetry, 60–80% of racemic compounds crystallize in either the $P2_1/c$, $C2/c$, or $P1$ space groups [10]. The most common group is $P2_1/c$, which belongs to the monoclinic system, and whose unit cell contains two each of the oppo-

site enantiomers related to one another by a center of symmetry and a binary screw axis.

A chirality classification of crystal structures that distinguishes between homochiral (type A), heterochiral (type B), and achiral (type C) lattice types has been provided by Zorkii, Razumaeva, and Belsky [11] and expounded by Mason [12]. In the type A structure, the molecules occupy a homochiral system, or a system of equivalent lattice positions. Secondary symmetry elements (e.g., inversion centers, mirror or glide planes, or higher-order inversion axes) are precluded in type A lattices. In the racemic type B lattice, the molecules occupy heterochiral systems of equivalent positions, and opposite enantiomers are related by secondary lattice symmetry operations. In type C structures, the molecules occupy achiral systems of equivalent positions, and each molecule is located on an inversion center, on a mirror plane, or on a special position of a higher-order inversion axis. If there are two or more independent sets of equivalent positions in a crystal lattice, the type D lattice becomes feasible. This structure consists of one set of type B and another of type C, but it is rare. Of the 5,000 crystal structures studied, 28.4% belong to type A, 55.6% are of type B, 15.7% belong to type C, and only 0.3% are considered as type D.

A detailed discussion of crystal packing and the resulting space groups has been given by Kitaigorodskii [13]. This approach assumes that molecular crystals are assemblages for which compactness tends toward the maximum that is compatible with the molecular geometry. He defined a packing coefficient as vZ/V , where v is the volume of the molecule, V is the volume of the cell, and Z is the number of molecules in the unit cell. In this way, the space-filling or packing coefficient in crystals always lies between 0.65 and 0.77, which is of the same order as the regular packing of spheres or ellipsoids. Molecules that owing to peculiarities of their geometry cannot attain a packing coefficient at least equal to 0.6 do not crystallize and can only form glasses from the melt. In order to fill space in the most compact manner with objects of indeterminate geometry, planes must be filled in a compact fashion. There are only a limited number of two-dimensional arrays in which an object can reside in contact with six neighbors, which is necessary for the optimal packing of a molecular assembly. It has been concluded that the binary screw axis is highly conducive to efficient

packing. In the final analysis, the limited number of possible combinations of stacking leads to a limited number of space groups they fulfill the requirements of three-dimensional close-packing [13].

The close-packing criterion for crystal stability generally yields lower free energies for a structure composed of a racemic assembly over that composed of homochiral molecules. This view has been expressed in the empirical rule of Wallach [14], which states that the combination of two opposite enantiomers to form a racemate is accompanied by a volume contraction. The many exceptions to this rule have been discussed in a systematic manner [15]. The modification to Wallach's rule contributed by Walden [16] is more generally valid; it states that if an enantiomer has a lower melting point than its corresponding racemate, then the crystals of the latter will have the higher density.

III. RELATIONSHIP BETWEEN MACROSCOPIC AND MICROSCOPIC STRUCTURES AS ILLUSTRATED BY QUARTZ AND ITS NONSUPERIMPOSABLE HEMIHEDRAL CRYSTAL FACES

The most appropriate point to begin a discussion of molecular and crystallographic chirality is to discuss the case of quartz. The holohedral morphology of a quartz crystal consists of a hexagonal prism, capped at each end by a hexagonal pyramid. This geometrical figure is highly symmetrical, possessing 24 symmetry elements of various types. It was noted by Hauy at the beginning of the nineteenth century that quartz crystals contained many small facets that considerably reduced the overall symmetry [17]. These facets were found only on alternate corners of the crystal and were therefore described as being hemihedral. As shown in Fig. 1, the distribution of these hemihedral faces gives rise to two forms of quartz, identified at the time as being either left- or right-handed [18]. It was noted that the two forms of quartz were mirror images of each other, and that no amount of positioning or orientation permitted the superimposition of one form onto the other. For instance, on a right-handed crystal the *s* trigonal pyramid lies to the right of the *m* face, which is below the predominating positive rhombo-

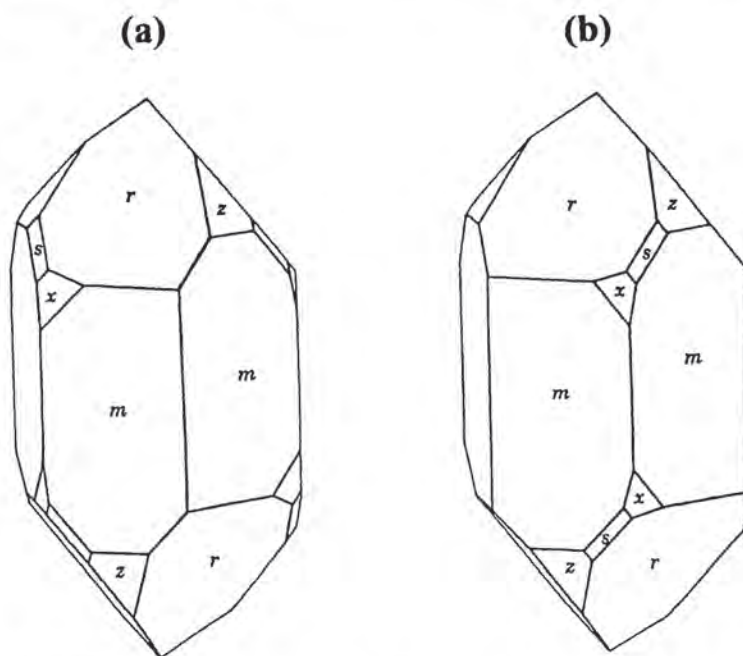


Fig. 1. Crystal morphologies of (a) left-handed quartz and (b) right-handed quartz. These are readily distinguished by the relative placement of the hemihedral facets. (The figure was adapted from Ref. 11.)

hedron *r*. On a left-handed crystal, the *s* trigonal pyramid lies to the left of the *m* face below the *r* rhombohedron.

The first complete determinations of the simple β -quartz structure were made by Bragg and Gibbs [19] and by Wyckoff [20], who reported that each silicon atom was surrounded by four oxygen atoms, two of which were above and two of which were below the silicon atom. The tetrahedral groups were found to arrange in spirals, each of which twisted in a definite manner. Thus all the spirals of left-handed quartz twist in one way, and all the spirals of right-handed quartz twist in the other way. The left-handed modification belongs to the enantio-

morphic space group $C3_12$, while the right-handed modification belongs to the mirror image space group $C3_22$.

Herschel investigated the optical properties of quartz slabs, which were cut perpendicular to the long crystal axis [17]. He found that crystals cut from left-handed quartz would invariably rotate the plane of linearly polarized light in a clockwise fashion, while crystals cut from right-handed quartz rotated the plane of linearly polarized light in a counterclockwise direction. Elsewhere Biot was performing extensive studies on the optical rotatory properties of certain compounds dissolved in fluid solutions, and it was at this time that the connection between optical activity and crystallographic properties was made.

Observations of this type were extremely important to Pasteur in that they permitted him to deduce requirements for chirality. He connected the concept of nonsuperimposable mirror images with the existence of chirality. Since quartz loses its optical rotatory power when dissolved or melted, he inferred that it was a helical arrangement of molecules in this particular solid that conveyed the given properties [17]. He also understood that solids could possess certain elements of symmetry and still exhibit optical activity (i.e., chirality) and therefore coined the term "dissymmetry" to describe materials whose mirror images were not superimposable on each other.

The twinning of quartz crystals can yield interesting structures. In the extreme case, the complete interpenetration of left- and right-handed structures can yield a crystal whose morphology is devoid of nonsuperimposable hemihedral faces (see Fig. 2a). As would be expected, no optical activity is observable from crystal slabs cut from this type of quartz structure [21]. Complete interpenetration would yield equal numbers of left-handed and right-handed spirals within the same crystal, with the optical effect of one type canceling the effect of the other. A more common occurrence is the so-called Dauphiné twin, which consists of a partial crystal interpenetration of differently handed quartz crystals (see Fig. 2b). In this latter instance one can find separate regions of opposite crystal chirality existing within the same twinned structure. In one sense, the instance of twinned quartz crystals can be considered to be a prototypical racemic mixture. The understanding of optical effects in quartz permitted Pasteur to develop impor-

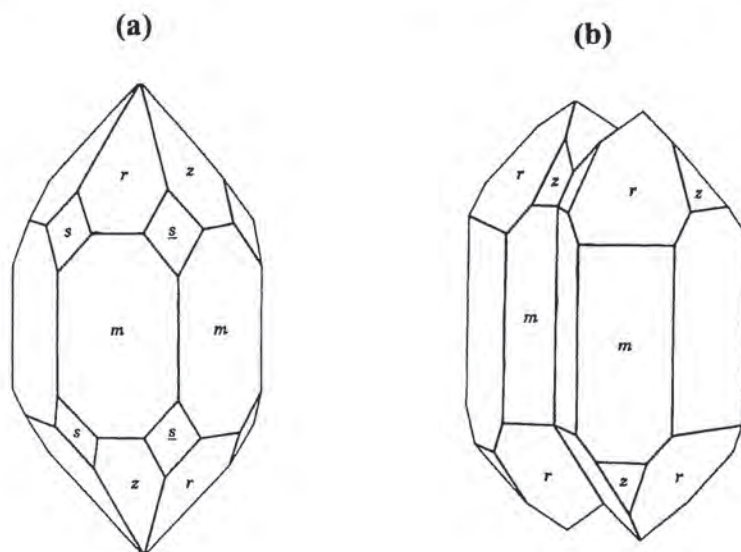


Fig. 2. Examples of twinned quartz crystals, illustrating (a) the lack of hemihedral facets in a completely interpenetrating crystal, and (b) the existence of oppositely handed quartz crystals in a single twinned structure. (The figure was adapted from Ref. 14.)

tant conclusions about the chirality of other crystal systems and in turn about individual molecules.

IV. RELATION OF MOLECULAR DISSYMMETRY AND THE CRYSTALLOGRAPHY OF TARTRATES

Crystalline quartz is one of those materials whose fundamental molecular units are achiral, but which are forced to adopt a chiral configuration by virtue of the crystal structure. The proof of this lies in the observation that quartz, when dissolved or melted, loses its ability to rotate the plane of linearly polarized light. The fusion/solubilization process

serves to destroy the spiral arrangement of the crystal, and the isotropic fluid that results contains no residual optical activity.

Early in the study of optical activity, it was learned that many organic compounds did not lose their ability to interact with polarized light even after being melted or dissolved in a solvent. A significant number of these optically active materials could be obtained as crystals that exhibited nonsuperimposable hemihedral crystal facets. Pasteur was the first to use the term "dissymmetry" to describe situations distinguished by the existence of nonsuperimposable mirror images. He understood that in the case of quartz, the dissymmetry was a result solely attributable to the nature of the crystal structure and that destruction of the structure eliminated the dissymmetry. He realized, however, that organic molecules that remained optically active even after dissolution or fusion must possess an inherent molecular dissymmetry. The remarkable nature of these postulates is that Pasteur made his proposals prior to the general acceptance of the tetrahedral nature of carbon valency.

The development of molecular dissymmetry as an understood property was inescapably linked to optical and structural crystallography [1]; tartaric acid and its salts provide the best illustrations. Tartaric acid was discovered by Scheele in 1769 in conjunction with wine-making, and numerous studies on its optical activity were carried out by Biot [17]. Perplexing the scientists of that era was a compound that became named racemic acid, whose name was derived from the Latin *racemus*, or grape. This compound was shown by Gay Lussac to be identical in composition with tartaric acid, though it differed significantly in its physical properties. For instance, the melting point of tartaric acid is 168–170°C, while the melting point of racemic acid is 206°C. The solid density of tartaric acid (1.760 g/mL) exceeds that of racemic acid (1.697 g/mL), as does the water solubility (1390 g/L for tartaric acid as compared to 206 g/L for racemic acid). Most important, Biot conclusively demonstrated that racemic acid was not optically active [17].

Pasteur studied the crystals of both tartaric and racemic acids and found that while tartrate crystals contained nonsuperimposable hemihedral facets, racemate crystals did not. Examples of the ideal crystal

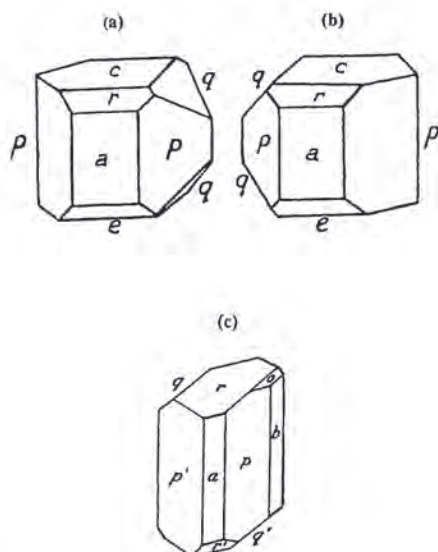


Fig. 3. Crystals of tartaric acid, obtained under conditions yielding the hemihedral facets distinctive of the (a) dextrorotatory and (b) levorotatory forms. Also shown is the (c) morphology associated with the holohedral modification of racemic acid. (The figure was adapted from Ref. 17.)

morphologies of these crystal forms are shown in Fig. 3. Pasteur subsequently proved the relationship between tartaric and racemic acids by mixing equal amounts of tartrate crystals having left-handed and right-handed hemihedrism and crystallizing out a tartrate substance whose crystal morphology was completely indistinguishable from that of racemate crystals isolated in the same manner [17].

While Pasteur was able to deduce many important conclusions using optical crystallography as his main tool, structural crystallography has the ability to go much further. It has been established that tartaric acid contains two centers of dissymmetry, with the naturally obtained optically active acid having the (*R,R*)-configuration [23]. En-

antiomerically pure tartaric acid crystallizes as an anhydrate phase in the monoclinic class (space group $P2_1$), with the unit cell containing two molecules [24,25]. The reported crystallographic data are $a = 7.715 \text{ \AA}$, $b = 6.004 \text{ \AA}$, $c = 6.231 \text{ \AA}$, and $\beta = 100.1^\circ$ [25].

Racemic acid has now been shown to consist of an equimolar mixture of (*R,R*)-tartaric acid and the unnatural (*S,S*)-enantiomer and is obtained as a monohydrate species. The compound crystallizes in the triclinic class (space group $P1$), with the unit cell consisting of one molecule of (*R,R*)-tartaric acid, one molecule of (*S,S*)-tartaric acid, and two water molecules [26]. This heterochiral unit cell is significantly different from the homochiral unit cell; $a = 8.06 \text{ \AA}$, $b = 9.60 \text{ \AA}$, $c = 4.85 \text{ \AA}$, $\alpha = 70.4^\circ$, $\beta = 97.2^\circ$, and $\gamma = 112.5^\circ$. The unit cell of racemic acid contains a center of symmetry, while that of tartaric acid does not.

The molecular conformation of the tartaric acid is not significantly different in the tartrate and racemate structures, but the different modes of molecular packing lead to the existence of the two very different crystal structures (see Fig. 4 for a comparison). For the tartaric acid anhydrate crystal, the molecular planes are held directly together by a complicated network of intermolecular hydrogen bonds [25]. The hydrogen bonding pattern in the racemic acid monohydrate crystal is very different: the molecules are bound into columns that are linked up to form sheets [26].

Another acid was occasionally encountered in the French wine-making industry that was chemically identical to tartaric and racemic acids but exhibited a completely different range of physical properties. It did not exhibit optical activity, nor could it be resolved into components that were themselves optically active [17]. The solid exhibited a melting point of $159\text{--}160^\circ\text{C}$, a density of 1.737 g/mL , and a solubility of 1250 g/L [22]. Pasteur was ultimately able to obtain quantities of this acid as a by-product from the heating of tartaric acid in the presence of basic substances.

It was learned that this nonresolvable, optically inactive form of tartaric acid was the meso form and that it contained two centers of opposite-sense dissymmetry in the same molecule. This compound has been found to crystallize in four distinct modifications, two of which are polymorphic anhydrates and two of which are polymorphic mono-

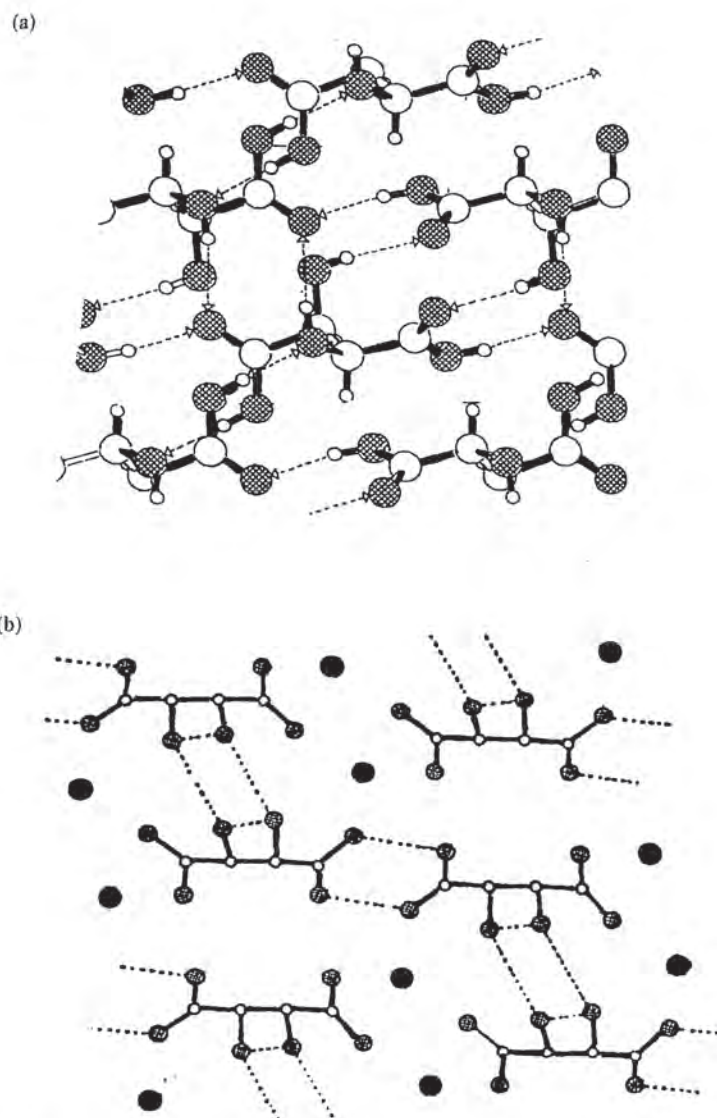


Fig. 4. Comparison of the molecular packing in crystals of (a) tartaric and (b) racemic acids. (The figure was adapted from data contained in Refs. 25 and 26.)

hydrates [27]. Triclinic *meso*-tartaric acid monohydrate crystallizes in the $P1$ space group with two molecules in the unit cell; $a = 5.516 \text{ \AA}$, $b = 9.220 \text{ \AA}$, $c = 7.330 \text{ \AA}$, $\alpha = 115.11^\circ$, $\beta = 93.62^\circ$, and $\gamma = 93.64^\circ$. The monoclinic monohydrate contains four molecules in the unit cell and crystallizes in the $P2_1c$ space group; $a = 5.215 \text{ \AA}$, $b = 5.019 \text{ \AA}$, $c = 25.92 \text{ \AA}$, and $\beta = 99.77.2^\circ$. The triclinic anhydrate crystallizes in the $P1$ space group with two molecules in the unit cell; $a = 9.459 \text{ \AA}$, $b = 6.464 \text{ \AA}$, $c = 5.396 \text{ \AA}$, $\alpha = 68.99^\circ$, $\beta = 76.36^\circ$, and $\gamma = 75.77^\circ$. Finally the orthorhombic anhydrate crystallizes in the $Pbn2_1$ space group with 16 molecules in the unit cell; $a = 19.05 \text{ \AA}$, $b = 9.88 \text{ \AA}$, and $c = 12.16 \text{ \AA}$.

In the triclinic *meso*-tartaric acid structures, the relative positions of most of the nonhydrogen atoms of the molecule are approximately equivalent to those of the corresponding atoms of racemic acid. As mentioned earlier, one of the important linkages in the racemic acid structure is a square system of hydrogen bonds between hydroxyl groups on four molecules, which forms columns parallel to the c -axis [26]. Analogous squares are formed in the *meso*-tartaric acid structures. In the structure of racemic acid, one carboxyl group of the molecule is directly linked with a carboxyl group of a centrosymmetrically related molecule, and the other carboxyl group is indirectly connected with a counterpart through water molecules. The first type of linkage is found at one side of the molecule in the triclinic anhydrate form of *meso*-tartaric acid, while the second type is found at one side of the molecule in the triclinic hydrate structure. In the monoclinic hydrate, one side of the molecule takes part in a carboxylic acid dimer configuration, but the linkage through water molecules at the other side of the molecule is a less common mutation of the hydrated links found in other cases. Evidently the geometry of *meso*-tartaric acid does not permit an ideal three-dimensional arrangement to exist with both types of intermolecular linkages present.

Undoubtedly, Pasteur's most widely known work in the area of molecular dissymmetry and crystallography came about during his study of the salts of tartaric acid [17]. In studies concerning the mixed 1:1 sodium/ammonium salts of tartaric and racemic acids, Mitscherlich had previously reported that both salts exhibited completely identical properties. This finding contradicted the growing body of evidence

that optically active and inactive forms of the same chemical species should exhibit different physical properties. As he expected, Pasteur found that sodium ammonium tartrate crystallized with the presence of nonsuperimposable hemihedral facets, but he was surprised to learn that crystals of sodium ammonium racemate contained nonsuperimposable hemihedral facets as well. Upon closer examination, Pasteur found that half of the crystals of sodium ammonium racemate exhibited hemihedral facets that spiraled to the left, while the other half exhibited hemihedral facets that spiraled to the right. He was able to separate manually the left-handed crystals from the right-handed ones and found that these separated forms were optically active upon dissolution. Drawings of these crystal habits are provided in Fig. 5.

The sodium ammonium salt crystallized from racemic tartaric acid has been found to crystallize in the orthorhombic $P2_12_12_1$ space group and contains four molecules in the unit cell [28]. This particular crystal class is noncentrosymmetric, and as a result individual crystals will be optically active. In fact, efficient growth of this tartrate salt only takes place if all the (*R,R*)-tartrate molecules crystallize in one ensemble of crystals, and if all the (*S,S*)-tartrate molecules crystallize in another ensemble. When formed below a temperature of 26°C, the preferred molecular packing does not permit the intermingling of the enantiomers to yield a true racemic crystal. The crystallization of sodium ammonium tartrate below 26°C results in a spontaneous resolution of the substance into physically separable enantiomers. Interestingly, a different polymorph forms above 26°C that requires a completely different packing pattern that allows for the formation of a racemic modification of sodium ammonium tartrate.

A series of half-neutralized salts of tartaric acid are also found to crystallize in the orthorhombic $P2_12_12_1$ space group (all containing four molecules in the unit cell) and are thus potentially resolvable by a mechanical separation. Ammonium hydrogen tartrate is found to crystallize with $a = 7.648 \text{ \AA}$, $b = 11.066 \text{ \AA}$, and $c = 7.843 \text{ \AA}$ [29]. Sodium hydrogen tartrate crystallizes with $a = 8.663 \text{ \AA}$, $b = 10.583 \text{ \AA}$, and $c = 7.228 \text{ \AA}$, while potassium hydrogen tartrate crystallizes with $a = 7.782 \text{ \AA}$, $b = 10.643 \text{ \AA}$, and $c = 7.608 \text{ \AA}$ [29]. Finally, rubidium hydrogen tartrate is found to crystallize with $a = 7.923 \text{ \AA}$, $b = 10.988 \text{ \AA}$, and $c = 7.653 \text{ \AA}$ [29].

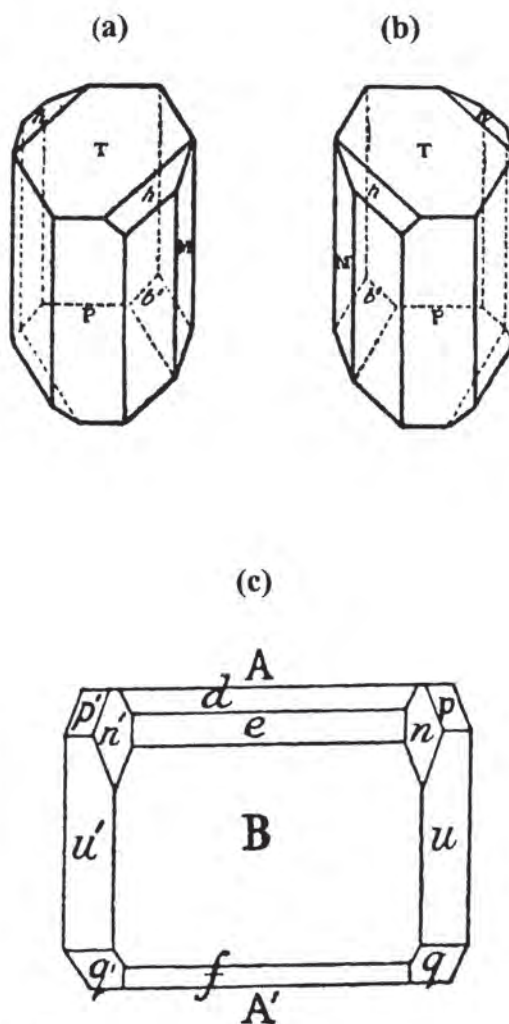


Fig. 5. Crystals of sodium ammonium tartrate, obtained under conditions yielding the hemihedral facets distinctive of the (a) dextrorotatory and (b) levorotatory forms. Also shown is the (c) morphology associated with the holohedral modification of sodium ammonium racemate. (The figure was adapted from Ref. 17.)

V. PHYSICAL CHARACTERISTICS OF RACEMIC MIXTURES OF DISSYMMETRIC SOLIDS

The early work of Pasteur clearly demonstrated the possibility that racemic compounds were mixtures that could be separated into their component enantiomers. It was found that while some compounds could be separated by direct crystallization, the majority required some type of chemical means to effect the separation. Compounds separable by the former method were termed *conglomerates*, while compounds separable only by the latter were termed *true racemates*. Much work has gone into the investigation of these two types of dissymmetric solids, and we will now consider some of the structural aspects of these systems.

A. Conglomerate Systems

Conglomerate solids are characterized by the presence of a single enantiomer within the unit cell of the crystal, even when the solid is obtained through crystallization of a racemic or partially resolved mixture. These solids consist of separate crystals, each of which consists entirely of one enantiomer or the other, which may be separated entirely on the basis of their physical properties. Compounds known to crystallize as conglomerates can be quite easily resolved, since the resolution step takes place spontaneously upon crystallization. The key to a successful resolution by direct crystallization lies in the means used to separate physically the crystals containing the opposite enantiomers. Jacques and coworkers have provided extensive summaries of the methods whereby direct crystallization can be used to effect the resolution of a racemic mixture [10,32].

The first method is the classical technique of Pasteur, which entails the mechanical separation of enantiomorphic crystals formed simultaneously while the mother liquor remains racemic. Enantiomer separation by this particular method is extremely time-consuming and is really impossible unless the crystals form with well-defined hemihedral faces. Nevertheless, it is often the method of choice to obtain the seed crystals required for other direct crystallization procedures. When a particular system has been shown to be a conglomerate, and the crys-

tals are not sufficiently distinct so as to be separated, polarimetry can often be used to establish the chirality of the enantiomer. Even a few seed crystals, mechanically separated, can be used to produce larger quantities of resolved enantiomerically pure material.

A second method of resolution by direct crystallization involves the localized crystallization of each enantiomer from a racemic, supersaturated solution. Oppositely handed, enantiomerically pure seed crystals of the compound are placed in geographically distant locations in the crystallization vessel, and they then serve as nuclei for the further crystallization of the like enantiomer. This procedure has been used to obtain both enantiomers of methadone, where approximately 50% total yield of enantiomerically pure material can be obtained [33]. An apparatus has been described that permits the automated use of localized crystallization, and it has successfully been used to separate the enantiomers of hydrobenzoin [34].

Enantiomer separation may be practiced on the large industrial scale using the procedure known as resolution by entrainment [35]. The method is based on the condition that the solubility of a given enantiomer be less than that of the corresponding racemate. To begin, a solution is prepared that contains a slight excess of one enantiomer. Crystallization is induced (usually with the aid of appropriate seed crystals), whereupon the desired enantiomer is obtained as a solid and the mother liquor is enriched in the other isomer. In a second crystallization step, the other enantiomer is obtained. The method can be applied to any racemic mixture that crystallizes as a conglomerate, and the main complication that can arise is when the compound exhibits polymorphism. In that case, the entrainment procedure must be carefully designed to generate only the desired crystal form.

Resolution by entrainment is best illustrated through the use of an example. Consider the bench scale resolution of hydrobenzoin [32]. 1100 mg of racemic material was dissolved along with 370 mg of (–)-hydrobenzoin in 85 g of 95% ethanol, and the solution was cooled to 15°C. 10 mg of the (–)-isomer was added as seeds, and crystals were allowed to form. After 20 minutes, 870 mg of (–)-hydrobenzoin was recovered. 870 mg of racemic hydrobenzoin was then dissolved with heating; the resulting solution was cooled to 15°C and seeded with 10 mg of the (+)-isomer. 900 mg of (+)-hydrobenzoin was recovered at

this time. The process was cycled 15 times and ultimately yielded 6.5 g of (-)-hydrobenzoin and 5.7 g of (+)-hydrobenzoin. Each isomer was obtained as approximately 97% enantiomerically pure.

Jacques and coworkers have provided compilations of the known conglomerate systems, for which approximately 250 organic compounds have been identified [36]. This relatively small number represents only about 10% of the number of chiral compounds for which resolutions have been published and this small fraction is seen as the major limitation to enantiomer resolution by direct crystallization. Nevertheless, it has been recognized that the formation of salts can greatly improve the odds of obtaining a conglomerate system, since one of the numerous possible salts of a racemate might exhibit the proper solid-state characteristics [37]. Substitution onto other portions of a compound can sometimes also yield a conglomerate system. For example, although mandelic acid and many of its derivatives crystallize as racemic mixtures, *ortho*-chloromandelic acid is obtained as a conglomerate [38]. Interestingly, both the *meta*- and *para*-chloromandelic acids crystallize as true racemates.

This seeming randomness of conglomerate formation has been explained through examinations of the thermodynamics of these systems. The stability of true racemic solids is defined by the free energy change associated with the process of combining the *R*-enantiomer with the *S*-enantiomer to produce the *R,S*-enantiomeric solid. This has been calculated to be in the range of 0 to -2 kcal/mol and is roughly proportional to the difference in melting points between the racemate and the resolved enantiomers [39]. In most cases, the free energy associated with the formation of racemates is exothermic, owing to the positive nature of the enthalpies and entropies of formation. In those cases where the melting point of the racemic mixture is at least 20 degrees lower than the melting points of the separated enantiomers, one generally obtains a conglomerate system.

Since conglomerate systems consist of totally independently formed enantiomer crystals and are therefore mere physical mixtures of the enantiomer components, these constitute a binary system. Such binary mixtures are easily described by the phase rule and can be profitably characterized by their melting point phase diagrams [10]. Since the components of a conglomerate racemate will melt indepen-

dently, the material behaves as if it were a pure substance. The melting point phase diagram will therefore exhibit a eutectic in its melting point phase diagram at the racemic composition. For a partially resolved conglomerate system, one can separate out the enantiomer present in excess simply by heating the sample to a temperature just above the melting point of the racemate and then collecting the crystalline excess of the residual enantiomer.

The liquidus line of a phase diagram can be calculated using the Schröder–Van Laar equation:

$$\ln X_i = \left(\frac{\Delta H^f}{R} \right) \left(\frac{1}{T_p} - \frac{1}{T_i} \right)$$

where X_i is the i th mole fraction of one of the enantiomers, ΔH^f is the molar heat of fusion of the pure enantiomer, T_p is the melting point of the pure enantiomer, and T_i is the melting point of the substance having mole fraction X_i .

An example of the type of melting point phase diagram that can be obtained for a conglomerate system is shown in Fig. 6, which illustrates one-half of the phase diagram reported for 4,4'-dimethyl-8,9,10-trinor-spiro-2,2'-bornane [40]. Below the eutectic temperature of 67°C, the system exists as a mixture of solid D-enantiomer and L-enantiomer. At the exact composition of the racemic mixture ($X = 0.5$), the system will exist entirely in the liquid phase above the eutectic temperature. At mole fractions where the amount of L-enantiomer exceeds that of the D-enantiomer, the system will exist as an equilibrium mixture of racemic liquid and solid L-enantiomer. As required by the phase diagram of a conglomerate, the eutectic temperature is the lowest temperature attainable at which any liquid phase can exist in equilibrium with any solid phase. An excellent fit of the data according to the Schröder–Van Laar equation was obtained, with $\Delta H^f = 5.9$ kcal/mol and $T_p = 95^\circ\text{C}$.

An alternative approach for the characterization of conglomerate systems is through the use of ternary phase diagrams, where one component is the solvent and the compound solubility is used as the observable parameter [10,32]. The racemic mixture is found to be more solu-

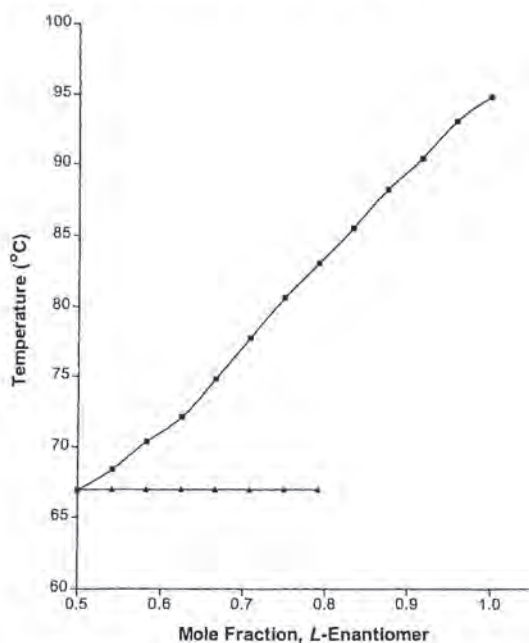


Fig. 6. Portion of the melting point phase diagram obtained for the crystalline conglomerate formed by 4,4'-dimethyl-8,9,10-trinor-spiro-2,2'-bornane. (The figure was adapted from data contained in Ref. 40.)

ble than are the separated enantiomers, and the rule of Meyerhoffer states that a conglomerate will have a solubility twice that of the resolved enantiomer [41]. This situation arises since ideally the mole fraction of an independent constituent in a liquid depends only on the enthalpy of fusion and melting point of the substance, and since according to the Schröder-Van Laar equation the solubility of one enantiomer cannot affect the solubility of the other. Jacques and Gabard have examined the solubilities of a number of conglomerate systems and

have largely confirmed the double solubility rule [42]. A selection of their results obtained for substances that are not dissociated in solution is presented in Table 1.

B. True Racemate Systems

True racemate solids are characterized by the presence of equimolar amounts of both enantiomers within the unit cell of the crystal. Materials of this type will crystallize within a centrosymmetric space group, which will necessarily be different from the space group of the separated enantiomers. As a result, the range of physical properties associated with a heterochiral solid must be completely different from those of the homochiral solid. For instance, the melting point of the exact racemic mixture may be greater than or less than that of the separated enantiomers; no general rule is available for a reliable prediction of behavior.

When a compound forms a true racemate, resolution by direct crystallization cannot be used to separate the enantiomers. Whether one works with melting point or solubility phase diagrams, one invariably finds that these systems yield diagrams that contain two minima. True racemates can therefore be separated after the performance of a derivatization reaction that alters the thermodynamics of the system so that the phase diagram now contains only a single minimum. In general, this minimum will not be located at the exact racemic composition but will instead be observed at some other concentration value. In fact, for the p-salt to be separated from the n-salt in a single crystallization step, the position of the eutectic should be substantially removed from the equimolar point. However, if the eutectic point should happen by accident to be close to the equimolar point, then the phase diagram would closely resemble that of a conglomerate. In that particular instance, it would be possible to devise an entrainment procedure for the separation of the p- and n-salts. This possibility has been demonstrated by the preferential crystallization of 4-methylpiperidinium hydrogen (*R,S*)-succinate [43].

The procedure most commonly used to alter the crystallization thermodynamics of true racemate systems involves the formation of dissociable diastereomer species [44]. These are most often simple salts

Table 1 Solubilities of Separated Enantiomers and Racemic Mixtures of Conglomerate Materials

Compound	System	Solubility of separated enantiomer (g/100 mL)	Solubility of racemic mixture (g/100 mL)	Ratio of racemate solubility to enantiomer solubility
Asparagine	water (25°C)	2.69	5.61	2.16
(α -Naphthoxy)-2-propionamide	acetone (25°C)	1.38	2.24	2.08
<i>p</i> -Nitrophenylamino propanediol	methanol (25°C)	1.63	3.35	2.14
<i>N</i> -Acetyl-leucine	acetone (25°C)	1.86	4.12	2.13
<i>N</i> -Acetyl-glutamic acid	water (25°C)	4.14	8.28	2.12
Diacetyldiamide	water (35.5°C)	12.4	23.7	2.14
3,5-Dinitrobenzoate-lysine	water (30°C)	6.96	13.17	1.99

formed between electron-pair donors and electron-pair acceptors. For instance, the first resolving agents introduced for acidic enantiomers were alkaloids, and hydroxy acids were used for the resolution of bases. The procedure has been known since the time of Pasteur, and extensive tables of resolving agents and procedures are available [45,46].

As an example, consider the case where a racemic acid is to be resolved through the use of a basic resolving agent. The first step of the resolution procedure involves formation of diastereomeric salts as in the table.

Racemic mixture	Resolving agent	→	Diastereomer salts
(+)-R-COOH	+ 2NH ₂ -R'(+)	→	(+)-R-COO-NH ₃ -R'(+)
(-)-R-COOH	+ 2NH ₂ -R'(+)	→	(-)-R-COO-NH ₃ -R'(+)

According to convention [47], the (*R,R*) and (*S,S*) diastereomers are termed the **p**-salts, and the (*R,S*) and (*S,R*) diastereomers are identified as the **n** salts. In the example used above, the (+)-R-COO-NH₃-R'(+)-diastereomer would correspond to the **p**-salt, and the (-)-R-COO-NH₃-R'(+)-diastereomer is the **n**-salt. In the usual practice, the **p**- and **n**-salts are separated by fractional crystallization, and the success of the resolution process is critically related to this crystallization step.

As mentioned earlier, the desirable effect of derivatization is to transform the undesirable thermodynamics of a true racemate system into those more amenable for direct separation of the diastereomers. For example, the enantiomers of phenylsuccinic acid can be separated after formation of the proline salt [48], and an example of the solubility phase diagram reported for the 1:2 phenylsuccinate/proline salt is shown in Fig. 7. In this work, it was found that the degree of resolution for the salt having the 1:2 stoichiometry was only minimally affected by temperature, but that separation of the 1:1 stoichiometric phenylsuccinate/proline salt was strongly affected by temperature. These findings illustrate the need for obtaining the phase diagram when designing enantiomeric separations, since the efficiency of the process can be strongly affected by environmental factors.

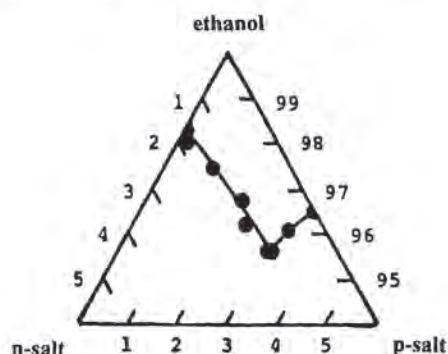


Fig. 7. Solubility phase diagram reported for the diastereomer system (1:2 stoichiometry) formed by ($\pm$)-phenylsuccinic acid and ($-$)-proline, using ethanol as the solvent system. (The figure was adapted from data contained in Ref. 47.)

For instance, the aqueous solubility of the n-salt of α -methylbenzylamine mandelate has been reported as 49.1 g/L, and the solubility of the p-salt as 180 g/L [49]. However, the relative solubilities of the p- and n-salt pairs is greatly affected by the choice of temperature or solvent system, and the pathway of the diastereomer separation can easily be manipulated by variations in experimental conditions. In fact, it is possible preferentially to crystallize either the p- or the n-salt depending on the range of parameters used.

The chiral discrimination existing in the diastereomer systems formed by ephedrine and various mandelic acids has been studied in great detail, using a variety of spectroscopic and structural tools. Although significant differences in the solubilities of the n- and p-salts obtained after reaction of ephedrine with mandelic acid were known to exist, both salts were found to crystallize in the same monoclinic space group with crystal structures that were isosteric [50]. In subsequent work, it was shown that the crystalline diastereomer salts formed by *R*-mandelic acid formed a more compact repeating pattern (with higher melting points and enthalpies of fusion) than did analogous salts

formed by *S*-mandelic acid [51]. It was deduced that subtle chiral discriminations led to the existence of different hydrogen-bonding modes, which in turn became manifest in a variety of other physical properties.

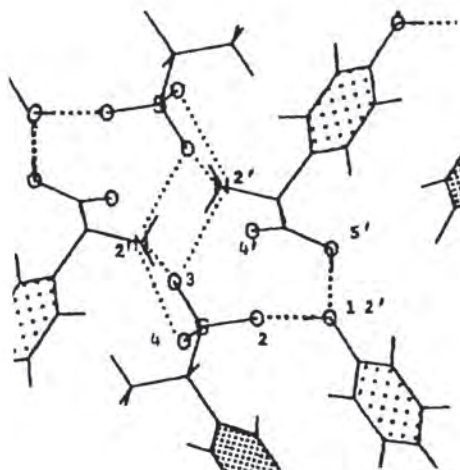
Distinctions in hydrogen-bonding networks were also noted during studies of the structures formed by α -amino acids and *S*-phenylethanesulfonic acid [52]. The differences noted for the two salts formed between *p*-hydroxyphenylglycine and the resolving agent are shown in Fig. 8. In another work, the diastereomers formed during the resolution of *R,S*-mandelic acid with *R*-2-*tert*-butyl-3-methylimidazolidin-4-one were characterized by varying networks of hydrogen-bonding, while the conformations of the molecular species were relatively equivalent [53].

Depending on the details of the lattice dynamics, it is certainly possible that the *p*-salt and the *n*-salt can crystallize in different space groups. For example, in the diastereomeric system formed by α -methylbenzylamine and hydratropic acid, the *p*-salt was found to crystallize in the $P2_1$ space group, while the *n*-salt crystallized in the $P2_12_12_1$ space group [54]. Although the molecular conformations in the two crystal forms were reported to be similar, the mode of packing to generate the individual crystals was different.

This latter behavior differs from that observed in the crystalline diastereomers of mandelic acid with 1-phenylethylamine [55]. Here the *n*-salt crystallizes in the triclinic $P1$ space group, while the *p*-salt crystallizes in the monoclinic $P2_1$ space group. The crystallographic structures of the two diastereomers reveal the existence of fairly equivalent hydrogen-bonding but substantially different conformations for the two molecular ions making up the donor-acceptor complex. These structural differences are manifest in the relative solubilities of the two diastereomers, where the aqueous solubility of the *p*-salt is much less than that of the *n*-salt.

Once either the *p*-salt or the *n*-salt (or both) is successfully crystallized, the diastereomer salt is usually dissociated and the resolving agent separated. In the specific instance of acid resolution, the diastereomeric salt is efficiently cleaved by means of a hydrolysis reaction as in the table.

p-salt



n-salt

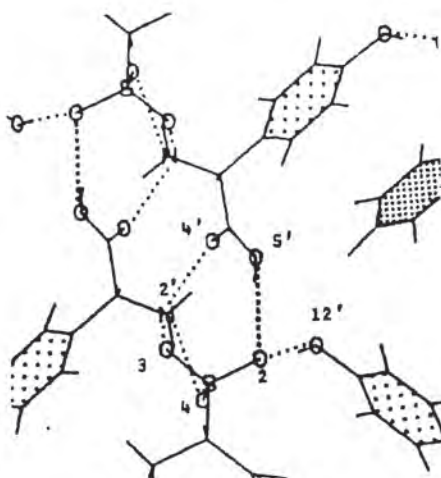


Fig. 8. Details of the hydrogen bonding existing in crystals of the two salts formed between *p*-hydroxyphenylglycine (HPG) and *S*-phenylethanesulfonic acid (PES). (The figure was adapted from data contained in Ref. 52.)

Separated diastereomers		Hydrolysis agent		Separated enantiomers
(+)-R-COO-NH ₃ -R'(+)	+	HCl	→	(+)-R-COOH + (+)-R'-NH ₃ Cl
(-)-R-COO-NH ₃ -R'(+)	+	HCl	→	(-)-R-COOH + (+)-R'-NH ₃ Cl

The diastereomer cleavage must be simple and selective, must take place in quantitative yield, must not racemize the resolved compound, and must leave the resolving agent in a form that is easily recovered. The recovery is usually effected by precipitation or extraction of the resolving agent. One important criterion in the choice of a resolving agent is the ease by which it can be dissociated and removed from the compound being resolved [44].

VI. SUMMARY

Observations on the chirality of crystals made it possible for Pasteur and others to identify dissymmetry as the true origin of optical activity. It became quickly evident that the molecular chirality associated with a given compound could be directly evident in the bulk crystallography of that compound. This in turn led to observable differences in a variety of physical properties, such as the melting point and the solubility of such species. Many chiral molecules have been observed to resolve spontaneously upon crystallization, forming enantiomorphic crystals that can be physically separated. Others can only be resolved through the formation and separation of diastereomeric species.

Technically, the crystallographic differences that exist between enantiomers and diastereomers do not fit the formal definition of polymorphism, since one may consider molecules of opposite dissymmetric senses (or mixtures of these) to be distinct chemical species. Nevertheless, such systems contain a number of features that are common to genuine polymorphic systems, and many of the same characterization techniques discussed in Chapter 5 of this book are equally appropriate here.

With more and more therapeutic agents being administered as

resolved enantiomers, methods for isolation of the desired dissymmetric species will be in constant development. Determination of the melting point or solubility phase diagram becomes a key element to the development process, since one can use this information to identify the agent in question as being either a conglomerate or a true racemate. Should the compound happen to crystallize as a conglomerate, then enantiomer separation by direct crystallization could represent the cost-effective route. Compounds identified as true racemates would by necessity need to be separated by the formation of dissociable diastereomers. Of course, when the compound in question can be synthesized directly with the proper enantiomeric composition, the need to conduct a resolution procedure must be critically examined. One should realize, however, that even when asymmetric synthesis is the chosen route, an investigation into the relationship between chirality and crystallography is essential to having an understanding of the molecular system.

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Impact of Polymorphism on the Quality of Lyophilized Products

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I. INTRODUCTION

Polymorphism in solids is a well-known phenomenon that, at least in principle, may dramatically impact both manufacturability and performance of a dosage form. Variation in the structure of the solid will cause variation in physical and chemical properties of the dosage form, and while such variations are not always of practical significance, a development scientist does need to be aware of the potential for polymorphism and the possible consequences. Awareness is generally high when dealing with tablet and/or capsule dosage forms, where the focus is often on manufacturability or on the impact of dissolution variations on bioavailability. The potential for significant polymorphism issues with lyophilized (freeze-dried) dosage forms is frequently ignored. It is not uncommon to see the procedure for preparation of a freeze-dried product described by one word, "lyophilized." In addition, the solid-state characterization of the freeze-dried product is frequently also described by the same word, "lyophilized." However, the implication that "lyophilized" is a satisfactory description of both the process and the resulting product is a gross oversimplification. The "phase chemistry" in freeze-drying is exceedingly complex, and stability problems are common (particularly with protein products). Depending on the formulation, process, and details of storage, changes in structure of the solid occur, often with dramatic consequences. Furthermore, the freeze-drying process is often long and expensive, and the impact of crystallization may change the processing time by an order of magnitude.

While polymorphism issues conventionally focus on different crystal forms of the same compound, the major question for freeze-dried products is normally whether the solid phases are crystalline or amorphous. The question of which crystalline polymorph of a given compound forms is usually of secondary importance.

While crystallization during freeze-drying may be of no practical consequence, there are a number of scenarios for which crystallization can have major impact on design of the process and/or performance of the drug. Altered stability is perhaps the most common result of crystallization. Crystallization of the drug will nearly always produce a significant stability enhancement, often by more than an order of magnitude [1,2]. Conversely, crystallization of one of the excipients will

often result in a major loss of stability, particularly when crystallization of buffer and/or crystallization of a stabilizer takes place in a protein formulation [3]. Since the amorphous form has greater solubility than any of its crystalline forms, crystallization of a sparingly soluble drug may prolong reconstitution time or even lead to incomplete dissolution at the intended reconstitution volume.

Crystallization can also have a major impact on design of the process. The impact may be positive or negative, depending on what crystallizes and when crystallization occurs. Since the glass transition temperature T_g' of the freeze concentrate depends upon the composition of the freeze concentrate, crystallization of one or more formulation components alters the composition of the solute phase(s) and thereby changes the value of T_g' . An increase of T_g' , as would be produced by crystallization of most inorganic salts, would allow the positive result of freeze-drying at a higher temperature. Conversely, a decrease in T_g' would require freeze-drying at a lower temperature, which would mean longer processing times and a negative outcome. Mannitol, perhaps the most commonly used excipient, is a useful bulking agent, but it can lead to vial breakage problems upon crystallization. While a bulking agent should crystallize during the freeze-drying process, mannitol crystallization should take place during freezing (not during the primary drying), or else significant vial breakage may occur [4].

The objective of this chapter is to analyze situations where crystallization may occur, and where one would anticipate a high probability of negative impact on product quality and/or process design. Following a summary of methodology for detection of crystallinity and a brief review of the freeze-drying process, a number of case histories will be described to illustrate the major generalizations that can be made regarding crystallization during freeze-drying and the impact of such crystallization.

II. DETECTION OF CRYSTALLINITY

Crystallinity in the freeze-dried product can normally be detected by polarized light microscopy, through the presence of birefringence in the sample [1]. Although microscopy is simple, quick, and sensitive to

small levels of crystallinity, it does have several disadvantages. Cubic crystals are isotropic and therefore nonbirefringent. Very small crystals may escape detection, and liquid crystal phases may be mistaken for three-dimensional crystallinity.

Differential scanning calorimetry (DSC) can also be used effectively in crystallinity studies. A sharp endotherm near the known melting point of a given polymorph is a clear signal of crystallinity arising from that polymorph. Further, when suitable calibration standards exist, the degree of crystallinity in a one-component system can be estimated from the areas under the melting endotherms (i.e., the heats of fusion) for the unknown and the standard. Conversely, a DSC scan of a sample containing a glassy phase produces a sharp increase in heat capacity at the glass transition. While enthalpy recovery effects may obscure the glass transition [5,6], enthalpy recovery can be eliminated by scanning slightly past the thermal event, cooling, and immediately rescanning. Enthalpy relaxation effects that produce the enthalpy recovery endotherm are irreversible on the time scale of the DSC scan, and the second scan will ordinarily reveal the glass transition. However, newer DSC techniques, such as "modulated DSC" [7] or "dynamic DSC" [8], have the potential for more efficient separation of enthalpy recovery effects from the glass transition. By superimposing a nonlinear temperature variation on the linear temperature scan rate, these methods can separate the reversible increase in heat capacity at the glass transition from pure kinetic effects, such as enthalpy recovery.

Degrees of crystallinity can also be evaluated from heat of solution measurements that compare the sample to be characterized with known crystalline and amorphous standards [1]. The heat of solution technique is particularly useful for materials that decompose upon melting. The method sensitivity depends upon the difference in heat of solution between crystalline and amorphous phases, and it can be better than $\pm 1\%$, allowing detection of small differences in highly crystalline samples [1].

X-ray powder diffraction is perhaps the "gold standard" for the qualitative determination of crystallinity. Not only can the presence of a crystalline phase be confirmed, but since each polymorph produces

a unique diffraction pattern, the question of which polymorph crystallized can be addressed. While x-ray powder diffraction requires expensive specialized equipment not likely to reside in a freeze-drying laboratory, such equipment is widely available in materials characterization laboratories. The experiments can be done quickly, and the results are normally unambiguous. One significant disadvantage is that very low degrees of crystallinity (i.e., much less than 10%) are frequently not detectable. An ensemble of techniques is often needed, such as the combination of x-ray powder diffraction and polarized light microscopy or DSC.

The preceding discussion was directed at the determination of crystallinity in a freeze-dried product, which from a quality control viewpoint is the most important material to characterize. However, from a research viewpoint it is often desirable to determine just where in the process crystallization does occur. Crystallization during freezing can easily be studied using variations of several of the techniques described above. Polarized light microscopy studies can be carried out with a freeze-drying microscope apparatus [9,10]. DSC studies on frozen solutions are routine, and at least at high solute concentration, glass transitions from an amorphous phase are easily recognized. In addition, a crystalline solute provides a eutectic melting endotherm that, if well removed from the main ice melting endotherm, is easily detected. Recently, x-ray diffraction has been used to characterize crystallinity in frozen systems [11]. This extension of conventional x-ray powder diffraction has great promise in studying complex crystallization problems directly relevant to freeze drying. However, all the methods for studying crystallization during freezing do have one limitation. It is well known that crystallization phenomena are sensitive to the thermal history of the sample (i.e., the cooling rate) and the volume of the sample. None of these laboratory techniques mimic either the thermal history or the sample volume of a product freezing in a freeze-dryer. Freezing in a vial is slower, it frequently involves less supercooling of water, and the volumes normally employed are far greater. In general, solute crystallization proceeds more readily in a vial. While special thermal treatments applied after the first freezing process (i.e., annealing) can be employed in an attempt to mimic freezing in a vial, some caution

must be exercised in the extrapolation of the specialized studies of crystallization to behavior in a vial.

III. THE FREEZE-DRYING PROCESS: THE IMPORTANCE OF TEMPERATURE CONTROL

Freeze-Drying is a drying process by which a solution (usually aqueous) is first frozen and then subjected to removal of the ice by direct sublimation during the stage of the process called primary drying. As a sample is cooled during the freezing process, the solution normally supercools to a temperature about 10–20°C below the equilibrium freezing point where ice nucleates and crystallizes. Once crystallization begins, the product temperature rises rapidly to near the equilibrium freezing point, decreases slowly until most of the water has crystallized, and then decreases sharply to approach the shelf temperature. The process of ice formation is accompanied by an increase in solute concentration between the growing ice crystals and an increase in solution viscosity. Solutes that tend to crystallize easily from aqueous solution, such as sodium chloride, buffer salts, and some low molecular weight drugs, may crystallize. Generally the drug itself and most carbohydrate excipients do not crystallize. Rather, they remain amorphous, and at the end of the freezing process they exist in a glassy state containing a relatively large amount of unfrozen water (i.e., approximately 20% w/w). The water that does not freeze is removed by desorption during the secondary drying stage of the process. Both primary drying and secondary drying are conducted at low pressure, typically in the range of 50–300 m Torr. Processing temperatures are low for primary drying (–10 to –40°C) and normally above ambient in the secondary drying step (25°C to 50°C). The objective is to produce a solid that will lead to the observation of a satisfactory storage stability for labile drugs. The resulting product is normally a parenteral product and may be a small molecule or biopolymer. Since the physical state of the product is sensitive to the product temperature history, it follows that product temperature control is critical, particularly in freezing and primary drying.

The rate of primary drying increases sharply with increasing prod-

uct temperature (i.e., roughly by a factor of 2 for a 5°C increase in product temperature). Therefore an efficient process is one that runs at a high product temperature. However, the temperature must not be too high or the product quality can be compromised. Ideally, the freeze-dried solid should be a porous cakelike structure with dimensions roughly equal to the frozen solution. Another way of stating this is that freeze-drying should proceed with retention of structure. Freeze-drying with retention of structure demands that one freeze-dry from a solid system. In a system where at least one solute component is crystalline, primary drying must be conducted below the eutectic temperature. As most drugs and excipients either have high eutectic temperatures or do not crystallize, eutectic melt is normally not a practical issue. Systems containing high levels of NaCl are an exception, as this salt exhibits a eutectic temperature of -21°C. In a system where the solute does not crystallize, primary drying must be carried out below the collapse temperature. Collapse is the amorphous system analog of eutectic melting, which is caused by the product temperature exceeding that of the glass transition temperature of the solute phase. If a product is being dried significantly above the glass transition temperature of the solute phase, the solute has sufficient fluidity to undergo viscous flow when ice is removed from that region of the sample. This phenomenon will lead to a loss of cake structure. Collapse of the cake means a loss of pharmaceutical elegance, higher residual moisture at the end of the freeze-drying process, possible increase of the reconstitution time, and possible crystallization of some components. Loss of activity can also occur with some protein formulations [12].

IV. CRYSTALLIZATION CASE HISTORIES

A. Impact of Crystallization on Process Design

1. *Crystallization and Collapse*

The collapse temperature depends upon the composition of the amorphous phase, and it has been found that crystallization of one or more components can significantly alter the collapse temperature. For example, human growth hormone (hGH) formulated with glycine and mannitol in a hGH: glycine:mannitol weight ratio of 1:1:5 can form a

completely amorphous system if frozen very quickly. Here, the collapse temperature is -24°C . However, a slower freezing rate permits crystallization of most of the mannitol, which results in a collapse temperature greater than -5°C [13]. Another example is provided by the glycine: sucrose system [14–16], where glycine is present in excess relative to sucrose. If frozen quickly, glycine remains amorphous, and the glycine: sucrose freeze concentrate has a very low collapse temperature (roughly -45°C , depending upon the exact composition). However, if glycine is allowed to crystallize, the glass transition temperature is essentially that of the sucrose freeze concentrate (about -34°C). Further, since the structure is maintained by the crystalline glycine, exceeding T_g' does not result in macroscopic collapse, and the system can be freeze-dried without apparent collapse (even at temperatures exceeding -10°C). Thus crystallization can transform a formulation from one that is nearly impossible to freeze-dry in a commercial operation to one where freeze-drying is relatively easy.

2. Mannitol Crystallization and Vial Breakage

Vial breakage is a common occurrence when freeze-drying mannitol solutions. This situation is particularly troublesome with high fill volumes, high mannitol concentrations, and low levels of other solutes. While in principle the stress created by a very rapid temperature change may be the cause, the fact that mannitol solutions are much more problematic than other formulations suggests that other factors dominate. It appears [4,17] that vials typically break during the early portion of primary drying as a result of mannitol crystallization. That is, when amorphous mannitol is created during freezing, the shelf temperature increase during early primary drying increases the product temperature above the T_g' (approximately -30°C), allowing rapid crystallization of mannitol at about -26°C and making the water that was part of the freeze concentrate free to crystallize. The resulting rapid volume increase is the stress that breaks the vials. As illustrated in Fig. 1, the probability of vial breakage depends significantly upon both the fill volume and the freezing procedure. A fast freeze to -50°C (which in reality is a typical commercial freezing procedure) results in about 40% of the batch being lost to vial breakage with the higher fill volume (5 mL). However, with a slow freeze to -18°C , no vial breakage is ob-

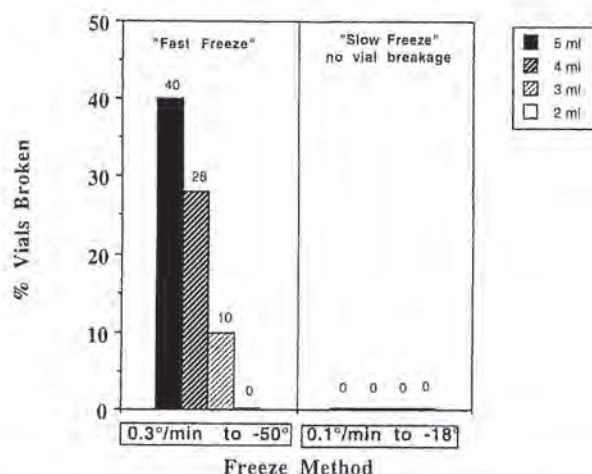


Fig. 1. Impact of process on crystallization: mannitol crystallization and vial breakage. Aqueous mannitol (5%) freeze-dried at a product temperature of -15°C during primary drying. (Data taken from Ref. 4.)

served. Vial breakage is also sensitive to the fill volume. No vial breakage is observed with the fast freeze procedure when the fill volume is only 2 mL.

The greater breakage with higher fill volumes is likely a direct result of mechanical effects. The key factor, however, is mannitol crystallization during freezing. If mannitol is induced to crystallize during freezing, mannitol crystallization occurs slowly from a fluid system without undue mechanical stress being developed. Using a slow cooling rate and limiting the product temperature to only -18°C during freezing (which is well above the T_g' temperature) produces crystalline mannitol at the end of the freezing process, thereby eliminating the rapid crystallization during early primary drying that is responsible for vial breakage.

3. Impact of Drying Process on Solubility

In some cases, a primary consideration in freeze drying is the production of the amorphous phase of the drug so that rapid and complete

solubilization is allowed. Dobutamine hydrochloride is an example where the room temperature equilibrium solubility of the crystalline form is marginal under the recommended reconstitution conditions. For this reason, its reconstitution is slow and often incomplete. Thus the drug is formulated with mannitol in a 1:1 weight ratio to produce highly soluble amorphous dobutamine hydrochloride. However, during early process development studies [18], it was noted that some batches produced a significant fraction of vials in which dissolution was incomplete. This finding was attributed to the presence of residual crystalline drug in batches that dissolved poorly. These batches also contained crystalline mannitol and crystalline drug in the solid state.

The results of one series of laboratory studies designed to explore the impact of process variations on crystallization are summarized in Table 1. In this work, the two process variations studied were stopper position and the criterion for judging the end of primary drying. Stopper position was judged a relevant variable due to the unfortunate fact that the 13-mm stoppers being used would frequently be seated too deeply into the vial, thereby restricting the vapor flow, raising the product temperature, and prolonging primary drying. The criterion for judging the end of primary drying was based upon product temperature response. The product temperature during primary drying normally varied from about -25°C early in primary drying to about -15°C at the end of primary drying. Therefore, once the product temperature sig-

Table 1 Impact of Process on Crystallization: The Effect of Drying Cycle on Crystallization of Dobutamine HCl

Stopper position	Product temperature when shelf $\rightarrow +40^{\circ}\text{C}$	#experiments (total # samples)	Percent crystallized
Normal	$+10^{\circ}\text{C}$ to $+20^{\circ}\text{C}$	3 (27)	0
1/2 to 3/4 closed	$+10^{\circ}\text{C}$ to $+20^{\circ}\text{C}$	2 (13)	38
Normal	$\leq +5^{\circ}\text{C}$	2 (13)	23
1/2 to 3/4 closed	$\leq +5^{\circ}\text{C}$	3 (22)	64

The formulation is 1:1 drug:mannitol. The freeze drying conditions are 100 mTorr chamber pressure and shelf temperature $+15^{\circ}\text{C}$, followed by $+40^{\circ}\text{C}$ when the product reaches the indicated temperature. Stoppers are 13 mm finish stoppers.

Source: Data taken from Ref. 18.

nificantly exceeded -15°C , the product temperature would rise sharply, thereby signaling the end of primary drying in that vial. In theory, the shelf temperature could be increased for secondary drying at this point without the risk of collapse. However, to allow for variation in primary drying times, and/or to allow for reduction of residual water in the "dry" product at low temperature, the shelf temperature is commonly held at the primary drying setting beyond the time at which all vials containing temperature sensors have completed primary drying. That is, only after this "operational" end of primary drying criterion has been met is the shelf temperature increased for secondary drying. Selection of this operational criterion is somewhat arbitrary. A process that specifies a longer hold time and/or a higher product temperature is more conservative in the sense that at the point of shelf temperature increase, the probability of a vial still containing ice is smaller and the residual water in the "dry" samples is less. Obviously, the more conservative process has the disadvantage of adding time to the process. Two operational criteria for the end of primary drying were used. The more conservative process yielded product temperatures in the $+10^{\circ}\text{C}$ to $+20^{\circ}\text{C}$ range before the shelf temperature was increased to 40°C . The more aggressive process yielded product temperatures less than 5°C at the point of shelf temperature increase.

The data shown in Table 1 clearly demonstrate the probability of crystallization and resulting insolubility increases as stoppers are positioned too deep into a vial. The "1/2 to 3/4 closed" term approximately represents a position such that 1/2 to 3/4 of the areas of the openings are closed. The operational criterion for the end of primary drying is also important, as the probability of crystallization is significantly higher for the more aggressive criterion of increasing the shelf temperature (i.e., a hold time giving measured product temperatures less than or equal to 5°C). It seems obvious that the higher product temperature and/or the prolonged primary drying time caused by improper stopper position, increases the probability of crystallization. It is possible that the aggressive criterion for ending primary drying resulted in some vials still containing ice when the shelf temperature was increased to 40°C , thereby yielding collapse and subsequent crystallization. However, the collapse temperature is relatively high (-10°C), and collapse was not visually apparent. It is more likely that crystallization occurred in product devoid of ice when the product temperature

exceeded the glass transition temperature of the moisture-rich solute early during secondary drying.

B. Buffer Crystallization and pH Shifts

It is ironic that while the function of a buffer system is to maintain constant pH, massive pH shifts during freezing may result from selective crystallization of one of the buffer components. In principle, and in practice, pH shifts often lead to degradation [19]. Under equilibrium conditions attained by seeding, the sodium phosphate buffer system shows a dramatic decrease of about 4 pH units due to crystallization of the basic buffer component, $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ [20]. Conversely, the potassium phosphate system shows only a modest increase in pH of about 0.8 pH unit. Under nonequilibrium conditions (i.e., no seeding) and with lower buffer concentrations, the degree of crystallization is less, and the resulting pH shifts are moderated [21]. Table 2 shows data accumulated during freezing of phosphate buffer solutions in large volumes at cooling rates intended to mimic freezing in vials [22,23]. For the concentrated buffer solutions (100 mM), the frozen pH values were close to the equilibrium values. However, lowering the buffer concentration by an order of magnitude considerably reduced the pH shift observed during freezing. It should also be noted that under some

Table 2 Shifts in pH During Nonequilibrium Freezing with Phosphate Buffer Systems

Concentration, mM	Initial pH	Frozen pH	ΔpH
Sodium phosphate buffer			
100	7.5	4.1	-3.4
8	7.5	5.1	-2.4
Potassium phosphate buffer			
100	7.0	8.7	+1.7
100	5.5	8.6	+3.1
10	5.5	6.6	+1.1

Source: Data taken from Refs. 22 and 23.

conditions, potassium phosphate buffers also exhibited large pH shifts during freezing. As shown in Table 2, if the initial pH is 5.5, the 100 mM potassium phosphate buffer increases in pH by 3.1 units during freezing.

Clearly, if drug stability is sensitive to changes in the solution pH, buffer crystallization must be avoided. In the author's experience, the best solution is to formulate so that the weight ratio of buffer to other solutes is very low [24,25]. The impact of other components on buffer crystallization and resulting pH shift is illustrated by the data in Table 3 [26]. As the weight ratio of sucrose to buffer increases, the pH shift on freezing moderates. At a weight ratio of 6:1, pH in the frozen material is identical to the starting solution pH. X-ray powder diffraction studies conducted on freeze-dried samples corresponding to the compositions given in Table 2 showed no evidence of crystallinity in the 6:1 weight ratio sample, which was consistent with the lack of a pH shift on freezing the 6:1 formulation.

Table 3 Impact of Formulation on Crystallization: The Effect of Sucrose on the pH Shift in Sodium Phosphate Buffer Systems During Nonequilibrium Freezing

Weight ratio, sucrose:buffer	Frozen pH (at -10°C)
0.0	4.1
0.36	4.2
1.0	4.6
2.0	5.0
6.0	7.5 ¹

¹ No crystallization of buffer observed in freeze-dried sample (x-ray powder diffraction).

The buffer concentration is 100 mM with an initial pH of 7.5.

Source: Data taken from Ref. 26.

C. Crystallization During Storage: Formulation, Water Content, and the Glass Transition

At least for materials that can crystallize, an amorphous form represents a metastable state, so thermodynamics requires that crystallization eventually occur. In general, however, crystallization of most materials is very slow until the storage temperature approaches or exceeds the glass transition temperature. However, crystallization well below the glass transition temperature does occasionally occur. Indomethacin crystallizes from the glassy state at a significant rate [27], and observations from the author's laboratory indicate that sodium cephalothin (a cephalosporin antibacterial) crystallizes very slowly at temperatures more than 100°C below the glass transition temperature. The degree of crystallinity was found to increase for relatively dry (approximately 1% water) freeze-dried sodium cephalothin (initially with partial crystallinity) after storage at -20°C after nearly 20 years. This behavior was observed even through the glass transition for "dry" sodium cephalothin is over 100°C.

Residual moisture has a profound influence on the rate of crystallization from the amorphous state [28-30]. As the moisture content increases, the glass transition temperature decreases sharply, and particularly for accelerated stability testing, it is not unusual for the glass transition temperature at modest levels of residual moisture to be less than the storage temperature. In such cases, increased molecular mobility in the amorphous material allows crystallization to take place on the timescale of the experiment (i.e., during storage). This phenomenon is illustrated by the water sorption isotherms shown in Fig. 2. As the relative humidity increases, the equilibrium water content increases smoothly until a moisture content is reached at which the moisture content suddenly decreases due to crystallization of the sugar. The moisture content at which the glass transition temperature of the sugar has been reduced to the sample temperature (25°C in these experiments) is denoted "Wg" on the plot. Note that crystallization does not occur on the rapid timescale of the experiment (i.e., several hours) until the moisture content is well above Wg. This indicates that rapid crystallization does not occur until the glass transition temperature has been reduced to well below the sample temperature.

While a well-designed freeze drying process can produce a prod-

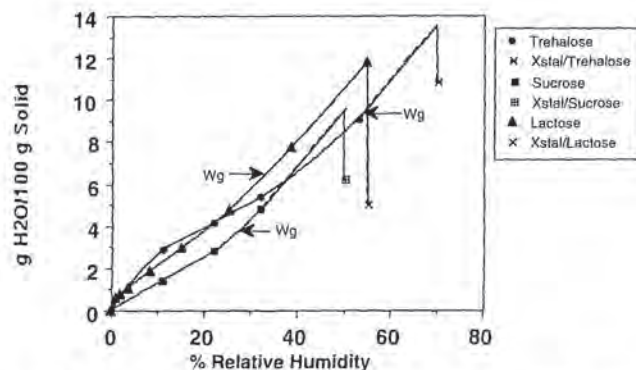


Fig. 2. The effect of water content on crystallization from the amorphous state: water sorption isotherms and crystallization of lactose, sucrose, and trehalose at 25°C. The equilibration time scale is hours. (Data taken from unpublished observations from this laboratory.)

uct of very low water content, and crystallization from the glassy state is not normally a practical problem at these low water contents, one often finds that the water content increases significantly upon storage. This can facilitate crystallization of one or more of the formulation components. The cause of the moisture increase is not moisture transmission from the ambient environment through the stopper but rather transfer of moisture from the stopper to the product [31]. Evidently, moisture is "pumped" into the stopper during steam sterilization, and while vacuum drying after steam sterilization and during the freeze-drying process removes water from the surface region of the stopper, water deeper in the interior is not completely removed. During storage, water slowly diffuses out of the stopper and absorbs into the dry product until equilibrium is reached. For the gray butyl rubber stopper studied, increases in water content of over 3% w/w were observed [31]. The level of water in the product is extremely sensitive to the method of vacuum drying the stoppers after their steam sterilization, with longer drying times leading to much reduced levels of moisture transfer. In addition, the equilibrium water content in the product depends on the mass of amorphous material in the vial, so that a small mass

leads to a high water content at equilibrium. Equilibrium is reached slowly at normal storage temperatures, and equilibration times are about 2 weeks at 40°C and 4–6 months at 25°C. While the moisture transfer problem can be moderated by heroic drying procedures after sterilization, a better solution is to use stoppers that do not release large amounts of water to the product. Such low-moisture stoppers are now available from some vendors.

The author has had experience with Daikyo Flurotech stoppers (available from the West Company), which produce a moisture increase of only about 0.1% under conditions where the butyl rubber stoppers produced an increase of over 1%. It is possible that the Flurotech coating (a fluorocarbon polymer coating) is responsible for the low moisture release properties. However, we have also found that Teflon-coated butyl rubber stoppers release moisture essentially in an equivalent fashion to stoppers of the same rubber formulation without the coating. Thus the low moisture properties of the Flurotech stoppers are more likely due to the rubber formulation.

It is important to note that, consistent with the impact of additional formulation components on buffer crystallization during freezing (Table 2), crystallization during storage is also retarded by the presence of additional solute components. The induction time for sucrose crystallization (Fig. 3) at 30°C (and 30% relative humidity) was studied as a function of level and type of saccharide additive [30]. Raffinose, lactose, and trehalose all significantly extend the induction time for crystallization, even at the relatively low levels investigated (less than 10%), with raffinose being the most effective of the three sugars studied. Since these additive sugars all exhibit glass transition temperatures in excess of 100°C when dry (compared with 75°C for sucrose), it is tempting to conclude that the mechanism involves an increase in the glass transition temperature. However, the actual mechanism may be more complex [30].

D. Stability Consequences of Crystallization

1. Crystallization of Drug

For nearly all compounds studied, the crystalline form has far greater stability than the corresponding amorphous form. This result is likely due to the crystalline form being able to provide an "inert" and rigid

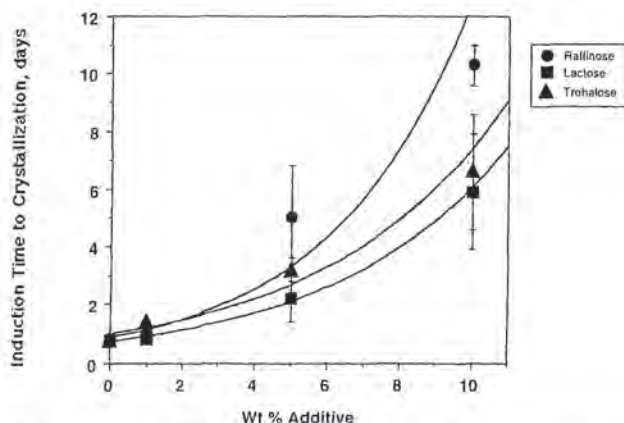


Fig. 3. Effect of formulation on crystallization: impact of saccharides on the crystallization of sucrose from the amorphous state. (Data taken from Ref. 30.)

environment for the reactive groups of a molecule. The few known "small molecule" examples of greater reactivity in the crystalline form arise from unusual details of crystal geometry where two reactive groups are forced in close proximity so that reaction requires little movement [32]. Insulin is the only known pharmaceutical example where greater reactivity is observed in the crystalline state relative to its amorphous phase [33].

The qualitative trend of degradation rate with crystallinity shown in Fig. 4 illustrates more typical behavior [1]. Here, the stability of cephalothin sodium clearly increases dramatically as the crystallinity increases. For this work, the degree of crystallinity was evaluated from the heat of solution of a given sample relative to the heats of solution of crystalline and amorphous standards. Although the instability of "hydrated" samples (i.e., in equilibrium with 31% relative humidity) is far greater than for corresponding dry samples, the qualitative trend of degradation rate with degree of crystallinity is the same. Many of the partially crystalline samples represented in Fig. 4 were prepared by freeze-drying, where significant differences in crystallinity arose

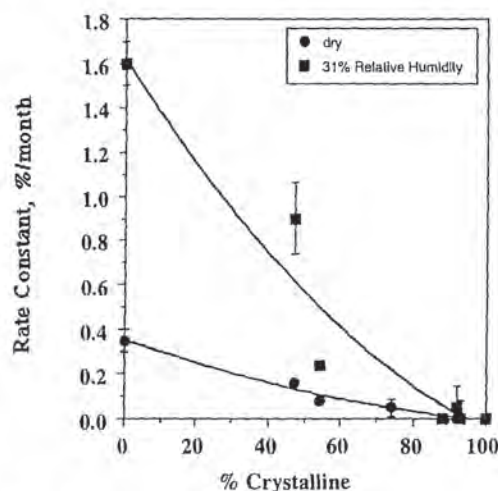


Fig. 4. Impact of drug crystallization on storage stability; effect of degree of crystallinity on the storage stability of cephalothin sodium at 50°C. The degrees of crystallinity were estimated from heat of solution data. (Data taken from Ref. 1.)

from both variations in process and inter-vial variations in crystallinity produced by nominally the same process [1]. Clearly, even with a given compound, crystallinity variations and corresponding stability variations can be quite striking.

2. Crystallization of Stabilizer

While crystallization of the drug increases stability, crystallization of an excipient intended to function as a stabilizer will decrease drug stability. To function as a stabilizer, the excipient must be in the same phase as the drug [24]. Complete crystallization of the stabilizer will lead to the existence of a simple physical mixture of an excipient and a drug (such as crystalline sucrose and glassy protein) and will not allow either molecular dispersion of the drug or a stabilizing interaction of drug and excipient. Thus the stability of human growth hormone in a system of amorphous glycine and mannitol is superior when compared to a formulation with crystalline glycine or mannitol [24].

An excellent example of the impact of stabilizer crystallization on protein stability during freeze-drying is provided by the series of mannitol/buffer formulations of β -galactosidase studied by Izutsu and coworkers [34]. Figure 5 illustrates the relative activity after freeze-drying as a function of the weight ratio of mannitol to phosphate buffer. As the weight ratio of mannitol to buffer increases, the stability also increases, and it reaches essentially 100% at a weight ratio of about 4.0. Interestingly, the stability decreased at higher weight ratios. It was noted that many of the systems contained crystalline material. Since systems without mannitol (where buffer crystallization might be expected) were amorphous, it appears that the crystalline phase was mannitol. In the figure, the data points referring to samples that were at least partially crystalline are given filled symbols. The vertical line at a weight ratio of 4.0 clearly separates the totally amorphous samples from the samples with crystallinity, and it also marks the region of maximum stability. As the mannitol content increases, stability in-

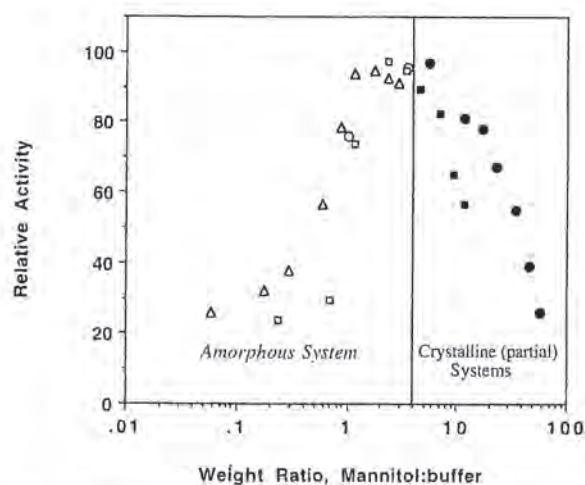


Fig. 5. Crystallization of stabilizer system and impact on enzyme stability during freeze drying; residual activity of β -galactosidase in mannitol:sodium phosphate formulations after freeze-drying. (Data taken from Ref. 34.)

creases as long as the mannitol remains amorphous. However, at sufficiently high amounts of mannitol, the substance crystallizes and removes much of the mannitol from the protein phase, thereby decreasing stability.

If a stabilizer crystallizes during storage, not only will the stabilizer be removed from the drug phase but also any moisture associated with the portion of the amorphous phase that crystallizes to an anhydrate will be shifted to the remaining amorphous phase (i.e., the drug phase), thereby increasing the moisture content of the drug and decreasing stability. Figure 6 illustrates the possible impact of this effect on stability. The data shown in Fig. 6 give estimated degradation rates at 5°C in a vinca alkaloid: monoclonal antibody conjugate formulated with a 1:1:1 weight ratio of conjugate/glycine/mannitol [25]. This formulation tends to crystallize during storage, producing a significant decrease in the glass transition temperature of the drug phase. Degradation rates, at least in systems above the glass transition temperature, were found to be strongly correlated with the difference between storage temperature and glass transition temperature. Degradation rates via hydrolysis of the vinca-antibody linkage (free vinca generation) and through vinca decomposition (largely oxidation) are shown as a function of water content for formulations that remain amorphous during storage and for samples where the glycine and mannitol excipients crystallize during storage. These data were projected from experimental observations at 25°C and 40°C at water contents of about 1% and higher. Due to sorption of water from the stopper, very low levels of moisture could not be maintained during storage. In Fig. 6, the circles with corresponding error bars represent direct experimental observation on samples where crystallization had occurred during storage. The close agreement between the projected data and the direct experimental observations provides some support for the accuracy of the extrapolation procedure used [25]. However, it must be recognized that the very sharp decrease in reaction rates projected below 1% water and the roughly fivefold increase in reaction rate upon crystallization are based upon the validity of correlation between reaction rate and $T-T_g$. Assuming that the projected data are at least semiquantitative, it is obvious that crystallization of excipient during storage has a serious stability consequence.

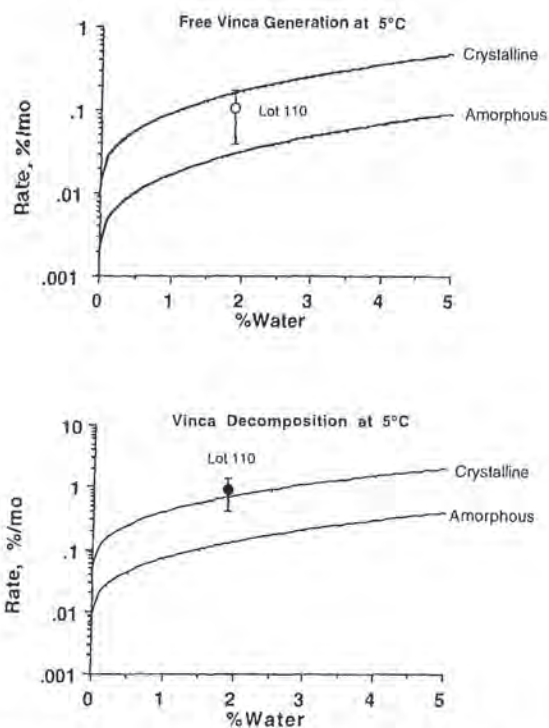


Fig. 6. Crystallization of stabilizer system and impact on storage stability: stability at 5°C of a KS1/4 monoclonal antibody:vinca alkaloid conjugate formulated with mannitol and glycine in a 1:1:1 weight ratio of conjugate:mannitol:glycine. Both mannitol and glycine crystallize early in storage. The smooth lines are data projected from 40°C and 25°C data for crystalline and amorphous excipient systems. The circles represent direct experimental observations at 5°C for "lot #110", which was "mostly crystalline" after storage. (Data taken from Ref. 25.)

A complex but informative example of the impact of crystallization on storage stability is provided by the effect of sucrose on the storage stability of ribonuclease A [35-37]. Figure 7 gives the percentage of initial enzyme activity remaining after storage for 105 days at 45°C for sucrose and Ficoll formulations of ribonuclease A (RNASE) buffered with 100 mM sodium phosphate (pH 6.4). With no excipient, the residual activity is about 60%, and the addition of increasing levels of Ficoll enhance stability to about 90% of initial. However, at low levels of added sucrose, the stability is sharply decreased, and only about 15% of initial activity remains at a level of 0.5% sucrose; but at higher sucrose levels, sucrose stabilizes at least as well as Ficoll. It was noticed that only the low sucrose content samples were brown in color, and they were similar in appearance to sucrose formulations that were freeze-dried from solutions at pH 3. The instability and color of the pH 3 samples were correlated with the onset of sucrose hydrolysis to the constituent reducing sugars. The interpretation of the data seems clear. At low sucrose levels, the sodium phosphate buffer crystallized,

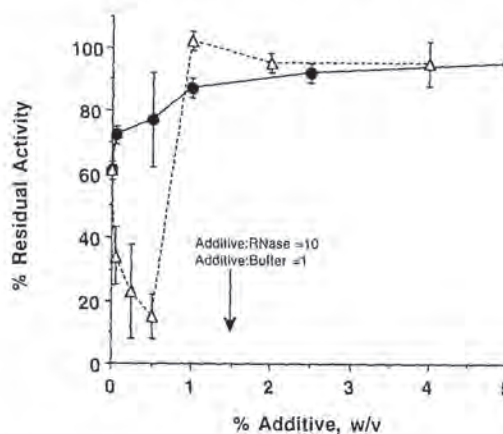


Fig. 7. The effect of formulation on storage stability of freeze-dried ribonuclease A: residual activity after storage for 105 days at 45°C. All formulations were freeze-dried from formulations containing 100 mM sodium phosphate buffer at pH 6.4. (Data taken from Ref. 35-37.)

producing a significant reduction in pH, thereby hydrolyzing the sucrose. The reducing sugars then qualitatively produced the same instability as was found with samples freeze-dried from pH 3 solutions. However, at higher levels of sucrose, buffer crystallization was reduced or eliminated, thereby circumventing sucrose hydrolysis.

V. SUMMARY AND CONCLUSIONS

A number of factors are critical for crystallization during or after freeze-drying. First, the nature of the solute is of obvious importance. Unless the solute will readily crystallize from an aqueous system at ambient temperatures, crystallization during freeze-drying is unlikely. Our experience suggests that if an aqueous solution crystallizes upon air drying on the laboratory bench, crystallization is possible in a freeze-drying process. Crystallization is obviously favored by higher concentrations of crystallizable solute. However, and most important, the presence of high levels of other solutes that will remain amorphous will generally impede or prevent crystallization of the potentially crystallizable solute. For example, while mannitol easily crystallizes from systems that are nearly pure mannitol, mannitol crystallization during freeze-drying is unlikely if it is present as a minor component. Crystallization during storage is sensitive to these factors, and it is also extremely sensitive to both residual water content and storage temperature. As a general rule, crystallization is rapid if the system is stored above the glass transition temperature. Nevertheless, with prolonged storage times, crystallization in some systems is possible even at temperatures well below the glass transition temperature.

Polymorphism may well be a significant problem with freeze-dried products. However, the issue of practical significance lies normally along the "amorphous vs. crystalline" pathway, rather than being concerned about which particular crystalline polymorph forms. In general, a crystalline drug implies better stability, but if crystallization of an excipient occurs, the result is normally inferior stability. Of course, if the drug is intrinsically stable in any form, then crystallization will have no stability implication. Finally, it should be emphasized poor inter- or intra-batch uniformity can result in systems where crystalliza-

tion is either erratic or partially present, leading to particular concerns for quality control groups. Again, the lack of uniformity in crystallization is only an issue if crystallization impacts some aspect of product quality.

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