International Journal of Pharmaceutics, 24 (1985) 1–17
Elsevier

IJP 00802

Research Papers

Evaluation of two concepts of crystallinity using calcium gluceptate as a model compound

R. Suryanarayanan and A.G. Mitchell

Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver V6T 1W5, B.C. (Canada)

(Received June 22nd, 1984)
(Modified version received October 26th, 1984)
(Accepted November 9th, 1984)

Key words: calcium gluceptate – models of crystallinity – quantitation of crystallinity; X-ray, calorimetry, density – effect of grinding on crystallinity – effect of crystallinity on solubility – effect of dehydration on solubility

---

Summary

According to the USP, solids are either crystalline, non-crystalline (amorphous) or a mixture of the two. The degree of crystallinity depends on the fraction of crystalline material in the mixture (two-state model). An alternative concept is that the degree of crystallinity has a value between 0% (amorphous) and 100% (perfect crystal) depending on the state of disorder in the lattice (one-state model). On grinding dehydrated calcium gluceptate (II) for increasing times, there were marked increases in apparent water solubility, decreases in the intensity of X-ray diffraction peaks, and heats of solution changed from endothermic to exothermic. The results are attributed to decreases in crystallinity, since surface area measurements showed that they could not be due to particle size reduction. Density values obtained for II using a liquid suspension method changed progressively with grinding. Lack of separation into two fractions on dispersion in the suspending liquid suggests that the decrease in crystallinity was not due to a decrease in the proportion of crystalline material in a crystalline-amorphous mixture. It is concluded that grinding decreases the crystallinity of II by increasing lattice disorder according to the one-state model, and thereby increases the apparent water solubility.

Correspondence: A.G. Mitchell, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Introduction

It is becoming increasingly recognized that the degree of crystallinity has an important bearing on the properties of pharmaceutical solids. Many properties such as chemical stability, water uptake and loss, solubility, dissolution rate, mixing, flow and compaction depend on the degree of crystallinity (Huttenrauch, 1978; York, 1983).

There appear to be two concepts regarding degree of crystallinity, and these are illustrated schematically in Fig. 1. According to the USP, solids are either crystalline, non-crystalline (amorphous) or a mixture of the two states (USP XX, 1980a); the degree of crystallinity depends on the fraction of crystalline material in the mixture. This can be called the two-state model. An alternative concept is that the degree of crystallinity has a value located on a continuous scale which varies between 100% (perfect crystal) and 0% (non-crystalline or amorphous) depending on the state of order/disorder in the lattice (Huttenrauch, 1978). Since there is no sharp distinction between the crystalline and amorphous states, this model is referred to as a one-state model. It is recognized that these two models represent, in a highly simplified way, the complex transition from the crystalline to the amorphous state and that other models or combinations of models are possible.

Powder X-ray diffraction is the method most widely used to determine the degree of crystallinity of pharmaceuticals (Black and Lovering, 1977; Nakai et al., 1977, 1982). A major limitation is the possible effect of particle size and sample orientation on X-ray intensity. Moreover, the separation of amorphous scattering from the total diffraction pattern is at best arbitrary (Alexander, 1969). Provided the energy difference between the crystalline and amorphous states is large, heat of solution measurements are more precise and less subject to artefacts than powder X-ray diffraction methods (Pikal et al., 1978). However, neither of these methods can

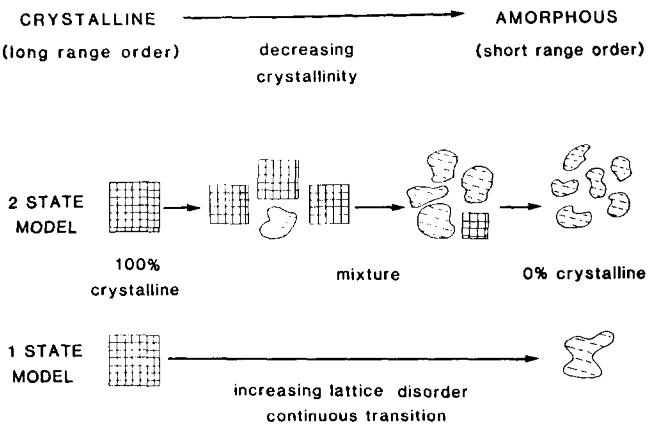

Fig. 1. Schematic representation of the two models of crystallinity. Squares represent the lattices of perfect crystals, while irregular shapes represent the amorphous state; no assumptions are implied regarding the structure of the latter.

distinguish between the two concepts of crystallinity, and in calculating the degree of crystallinity, the two-state model is implicitly assumed. Both X-ray line broadening and a decreased enthalpy of solution suggest a decrease in crystallinity but give no indication of the extent of lattice disorder of the individual particles in the sample under investigation.

The density of dislocations (line defects) provides some insight into the state of order of single crystals. Because of the localized energy associated with dislocations, two-dimensional nucleation occurs more rapidly at the site where a dislocation emerges on a crystal surface than elsewhere. Treating a cleaved surface with an etching solution reveals the dislocation sites as etch pits which can be seen and counted under a microscope (Burt and Mitchell, 1981; Friesen et al., 1981). This method has several limitations: (i) it is restricted to large well-formed crystals; (ii) only up to about 10^8 dislocations/cm^2 can be visually counted; and (iii) the method is restricted to quantitating dislocations; other types of crystal imperfections such as point defects are not included.

Density measurements can also provide an indication of the state of order of a solid. Although there are exceptions, crystalline materials in general have a higher density than their amorphous counterparts because the atoms in the crystal lattice are located at the minimum possible distance from each other. An increase in lattice disorder (decreasing crystallinity) will usually result in an increase in volume and therefore a decrease in density. Among the several techniques used to determine the density of solids, the suspension density method was selected because it is not only possible to differentiate between samples having very small differences in density (Johnston and Hutchison, 1940) but it may also provide a method of distinguishing between the two models of crystallinity. A liquid is chosen which has a density close to that of the solid and which neither reacts with or nor dissolves the solid. The solid is dispersed in the liquid and the temperature altered until the solid is suspended, at which temperature, the density of the solid is equal to that of the liquid. Since the temperature coefficient of expansion of a solid is generally much less than that of a liquid, the effect of temperature on the density of the solid is considered negligible (Estermann et al., 1949). If the simple two-state model is valid, then on dispersion in the suspending liquid, a partially crystalline sample would separate into two fractions as a result of the difference in density between the crystalline and amorphous states. On the other hand, if the one-state model is applicable, then progressive changes in crystallinity should be accompanied by gradual, progressive changes in density.

The suspension density method was used by Vaughan et al. (1958) to measure the decrease in density on compressing potassium chloride crystals, and by Huttenrauch (1978) to determine changes in the degree of crystallinity of microcrystalline cellulose and lactose on grinding, drying and compaction.

The objective of this work was to evaluate the two concepts of degree of crystallinity using calcium gluceptate as a model compound. Calcium gluceptate is official in the USP (1980b) as the calcium salt of D-glycero-D-guco-heptonic acid, which is the α-epimer of glucoheptonic acid. Calcium gluceptate was formerly available as an amorphous anhydrate which was very soluble in water, but the solutions were unstable, and the crystalline hydrate precipitated on storage (Suryanarayanan and Mitchell, 1981, 1984). Synthesis of amorphous calcium gluceptate has been impossible in recent years [1], and we have found that commercial material from a number of sources is a crystalline hydrate containing 3.5 molecules of water of crystallization. The crystalline hydrate was used in this work to investigate the effects of dehydration and grinding on increased apparent water solubility and decreased crystallinity.

Materials and Methods

Materials

Acetonitrile (Caledon), carbon tetrachloride (BDH), ethylene dibromide (BDH), hydrochloric acid (Allied Chemicals), lithium fluoride (Fisher), methanol (Fisher), methylene chloride (Caledon) and tris(hydroxymethyl)aminomethane (Parr) were used as received. Calcium gluceptate (Pfanstiehl, lot 12953-D; amorphous anhydrate) has been described previously (Suryanarayanan and Mitchell, 1984). Calcium gluceptate, (I), (Pfanstiehl, lot 13313-E; crystalline hydrate) was used as received or was dried at 60°C under vacuum (pressure < 130 Pa) for 16 h (USP 1980b), to give the anhydrate (II).

Gas-liquid chromatography (GLC)

The USP monograph for calcium gluceptate specifies calcium α-glucoheptonate, but the analytical procedures described in the USP for the identification and assay of calcium gluceptate do not distinguish between the calcium salts of α- and β-glucoheptonic acids. Using a GLC technique, Suryanarayanan and Mitchell (1984) have shown that some commercial samples of calcium gluceptate contain mixtures of the α and β epimers. The material used in this work was calcium α-glucoheptonate, and thus complied with USP specifications. GLC analysis of II, and II ground for 4 h, showed no evidence of degradation on drying or grinding.

Thermogravimetric analysis (TGA)

The weight loss on heating I up to 140°C at $10°\text{C} \cdot \text{min}^{-1}$ in a thermogravimetric analyzer (Du Pont 900/950) was 11.3% w/w.

Grinding

Both I and II were ground in a mechanical agate mortar and pestle (Pulverisette 2, Fritsch) for varying times.

Apparent solubility

Unground and ground samples of I or II were added to about 0.5 ml of distilled water in a culture tube and dissolved by vigorous shaking at room temperature

---

[1] Dr. A.G. Holstein, Pfanstiehl Laboratories, Waukegan, IL, U.S.A.; personal communication, 1980.

(~ 22°C) using a vortex mixer. At the first sign of persistent turbidity, the addition of solid was stopped and the solution weighed. The water was evaporated off at 60°C under vacuum until the residual solid reached a constant weight.

Surface area

About 100 mg of each sample was accurately weighed into the sample cell of a surface area analyzer (Quantasorb Sorption System, Quantachrome). The specific surface area was determined by the multipoint BET method (Lowell, 1973) using 0.072, 0.104 and 0.184 mol% krypton (adsorbate) in helium (carrier).

Polarized light microscopy

The samples were mounted in mineral oil and examined by means of a polarizing microscope (Standard 14, Zeiss). The birefringence and extinction positions of II ground for varying times were compared with unground II and the commercial amorphous anhydrate.

Determination of degree of crystallinity

Powder X-ray diffraction

The samples were exposed to Ni filtered CuKα radiation (35 kV × 15 mA) at a scanning rate of 1° $2\theta \cdot \text{min}^{-1}$ in a wide angle X-ray diffractometer (Philips) with a xenon proportional counter. Degree of crystallinity determinations were carried out only on ground samples of II. Around a 2θ value of 20°, II intensely diffracts X-rays, and the peak at 20.1°, which exhibited the largest deviation from baseline, was chosen for crystallinity calculations (Fig. 2a). Crystalline and amorphous standards are required for quantitative estimates of percent crystallinity. Unground II was chosen as the crystalline standard (100% crystallinity). After grinding for 4 h,

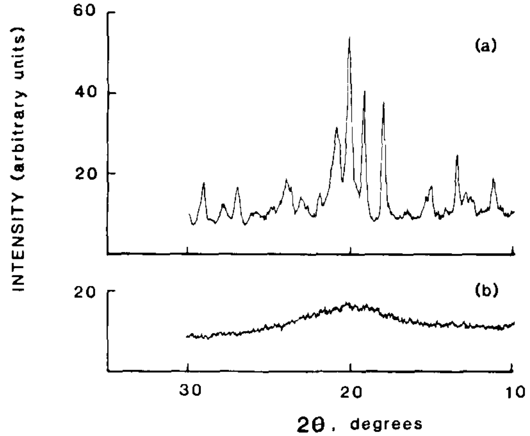

Fig. 2. Powder X-ray diffraction patterns of (a) II (100% crystalline standard), and (b) II ground for 4 h (0% crystalline standard).

II became amorphous to X-rays (Fig. 2b) and was used as the amorphous standard (0% crystallinity). Various proportions of the amorphous and crystalline standards were mixed to yield samples of known crystallinity from 0% to 100%. To an accurately weighed sample of each mixture, 11% w/w lithium fluoride was added as an internal standard, mixed well and the mixture redried at 60°C under vacuum to constant weight (Imaizumi et al., 1980). Lithium fluoride was chosen as the internal standard because the 2θ values at which it diffracts X-rays do not coincide with those of II. The ratio of the X-ray diffraction intensity of II at 20.1° 2θ to that of lithium fluoride at 45.0° 2θ was calculated for samples of varying crystallinity from 0 to 100%. This ratio was plotted as a function of percent crystallinity and used as a standard curve (Fig. 3). Fig. 4 is a representative X-ray diffraction pattern of a 20% crystalline sample prepared by mixing appropriate weights of the crystalline and amorphous reference standards.

To investigate the effect of grinding time on the degree of crystallinity, II was ground for varying periods of time. To an accurately weighed ground sample, 11% w/w lithium fluoride was added, the mixture was redried and the ratio of X-ray diffraction intensities determined as above. The degree of crystallinity at each grinding time was determined from the standard curve.

In an attempt to eliminate the effect of particle size on the intensity of diffracted peaks, the samples were initially sieved and a −100 +250 sieve fraction used for X-ray analysis. However, examination using a scanning electron microscope showed that after grinding, the powder consisted of aggregates of particles, and sieving was

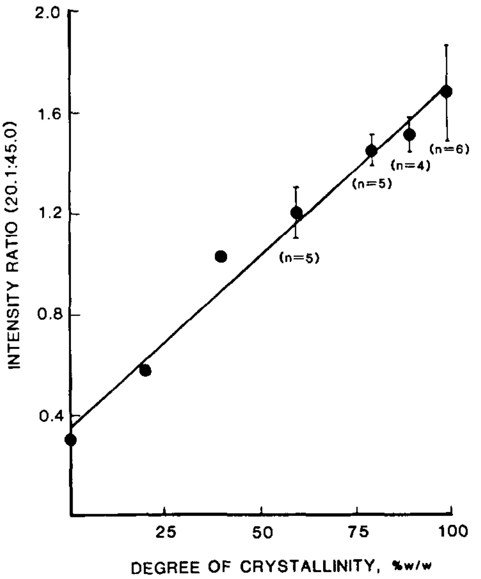

Fig. 3. Standard curve relating the degree of crystallinity of II and intensity ratio of the X-ray diffraction peak of II at 20.1° 2θ to that of lithium fluoride at 45.0° 2θ. Mean ±standard deviation are shown for selected values; other values are averages of two determinations.

discontinued. The intensity of the diffraction peaks was unaffected by the method of packing the powders into the sample holder, showing that the particles did not exhibit a preferred orientation.

Calculating the degree of crystallinity from an X-ray diffraction pattern usually involves measuring the total area under the curve and subtracting the contribution due to amorphous scattering. This method is arbitrary because of the inherent difficulty in separating the amorphous scattering from the crystalline diffraction. For example, Black and Lovering (1977) found that a digoxin sample judged to be crystalline by polarized-light microscopy was only 50% crystalline according to the above method of calculating crystallinity. They attributed the low value to overlap of crystalline peaks and proceeded to assume that the sample was 100% crystalline. The method of Imaizumi et al. (1980) successfully overcomes the problem by adding an internal standard and calculating intensity ratios rather than the areas under the curve. The effects of factors like background scattering need not be considered because the experimental crystallinity was determined from the standard curve (Fig. 3).

Solution calorimetry

Heats of solution were determined at room temperature (~ 22°C) using a solution calorimeter (Model 1451, Parr) with distilled water as the solvent. The energy equivalent of the calorimeter and its contents was determined by dissolving an accurately weighed amount of tris(hydroxymethyl)aminomethane in 100 g of 0.1 M hydrochloric acid and measuring the temperature change. The calorimeter was standardized as described previously (Suryanarayanan and Mitchell, 1984).

The crystalline and amorphous standards were mixed in various proportions to give samples of known percent crystallinity as before, and their heats of solution determined. A standard curve was plotted of the heat of solution as a function of percent crystallinity (Fig. 5). Samples of II ground for different times were redried at 60°C under vacuum to constant weight before their heat of solution values were measured and the percent crystallinities were determined from the standard curve. The final solution concentrations were normally less than 0.01 M.

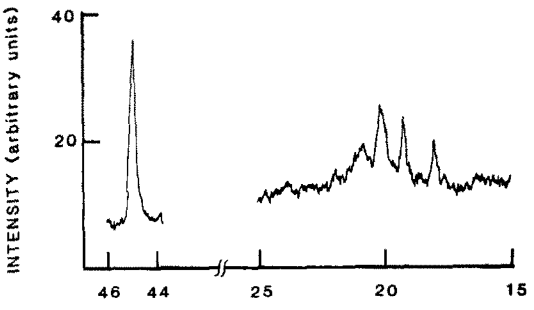

Fig. 4. Powder X-ray diffraction pattern of 20% crystalline II containing 11% w/w lithium fluoride as the internal standard.

Suspension density method

Carbon tetrachloride and ethylene dibromide were chosen as the suspending liquids because neither of them reacted with nor dissolved II. The density of carbon tetrachloride was lower than that of II, while that of ethylene dibromide was higher. The two liquids were mixed in varying proportions until a mixture was obtained with approximately the same density as II.

Density determinations were carried out on unground and ground samples of II. After grinding, the samples were dried at 60°C under vacuum to constant weight. Each sample was transferred to a Quantasorb sample cell, and a stream of nitrogen passed over it at room temperature for 4 h to dry the powder and remove surface impurities. The cell is automatically sealed on disconnection from the Quantasorb Sorption System so that the contents do not come in contact with the atmosphere. The cell was transferred to a 'dry chamber' (maintained at < 1% RH with phosphorus pentoxide). Here the solid was dispersed in the carbon tetrachloride–ethylene dibromide mixture contained in a borosilicate glass tube (Kimax, Owens-Illinois). The tube was closed tightly with a polytetrafluoroethylene-lined screwcap and transferred to a jacketed cell containing water (Fig. 6). Water was pumped from a thermostatically controlled water bath (Magniwhirl, Blue M Electric) through the double-wall of the cell and then through the outer jacket surrounding the oscillating tube of a digital density meter (Model DMA 45, Paar). The temperature of the water bath was altered until the dispersed sample was suspended. A sample of the pure liquid mixture was immediately injected into the oscillating tube of the density meter. After equilibration (density readings constant to ±0.0001), the density of the suspension liquid gave the density of the suspended solid. Before using the suspension cell to determine the density of samples of II, the density meter was calibrated

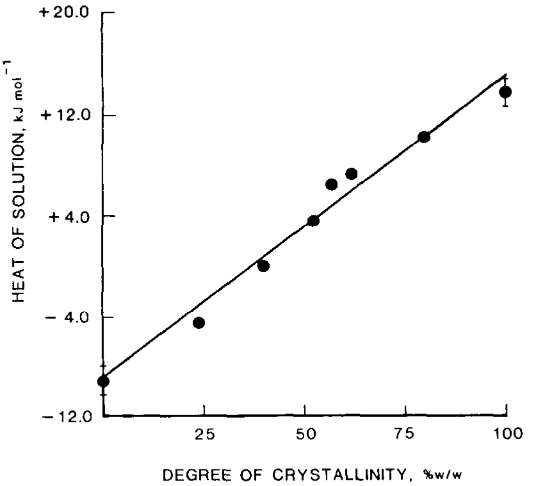

Fig. 5. Standard curve relating the degree of crystallinity and the heat of solution of II in water at room temperature (~ 22°C). The mean ±standard deviation of 100% and 0% crystalline standards (n = 4) are shown.

at 20°C with air and distilled water and its accuracy was checked by measuring the density of a number of liquids and comparing the experimental with the literature values. Experimentally determined density values (in $g \cdot cm^{-3}$) were: acetonitrile 0.7823 [0.7822 (Lange's Handbook of Chemistry, 1979)], methanol 0.7920 [0.7915 (Merck Index, 1983a)] and methylene chloride 1.3255 [1.3255 (Merck Index, 1983b)], which agree closely with the literature values given in parentheses.

The densities of the reference liquids were first determined with the flow of water in the direction shown in Fig. 6, then the direction of flow was reversed. The densities were independent of the direction of flow, showing that there was no significant difference in the temperature between the suspension cell and the density meter. The temperature of the water bath was accurately controlled to ±0.005°C by means of a proportional temperature controller (Model 76, YSI). Precise temperature control was necessary because a decrease in crystallinity from 100% to 0% caused a change in suspension temperature of only about 7°C. The density values of II ground for varying time periods were determined and the degree of crystallinity calculated from:

$$\% \text{ crystallinity} = \frac{\rho - \rho_a}{\rho_c - \rho_a} \times 100 \tag{1}$$

where ρ = density of sample under investigation, ρ_a = density of amorphous standard (II ground for 4 h), and ρ_c = density of crystalline standard (unground II).

Results and Discussion

According to the U.S.P. (1980b), calcium gluceptate is an anhydrate or an hydrate containing 2 molecules or 3.5 molecules of water of crystallization. The weight loss of I determined by TGA (11.3% w/w) is close to the theoretical weight loss of 11.4% w/w expected for the 3.5 hydrate. I had a low aqueous solubility of 0.07 molal, but

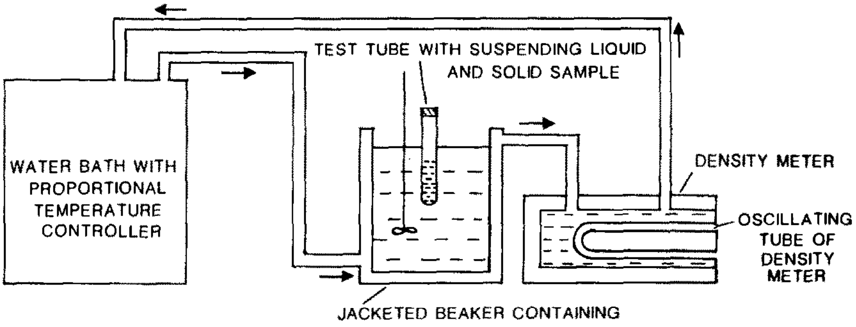

Fig. 6. Schematic diagram of apparatus for suspension density determinations. Arrows indicate the direction of water flow.

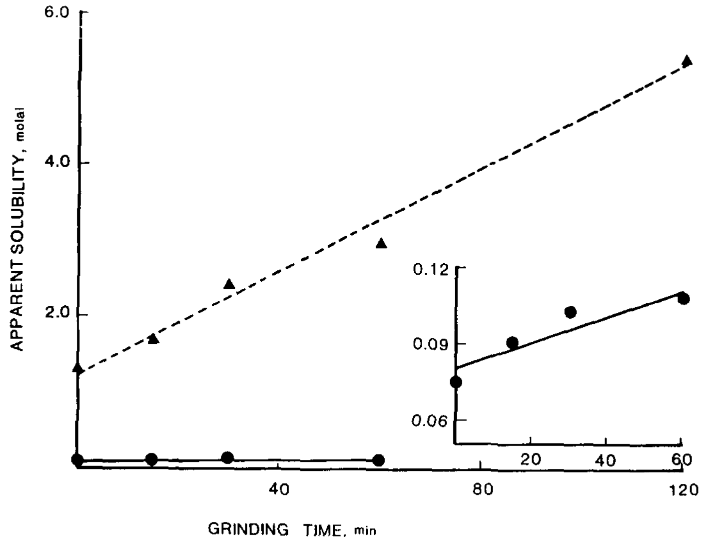

Fig. 7. Effect of grinding on the apparent solubility of I (●———●) and II (▲--▲) in water at room temperature (~ 22°C). Inset: solubility values of I plotted on an expanded scale.

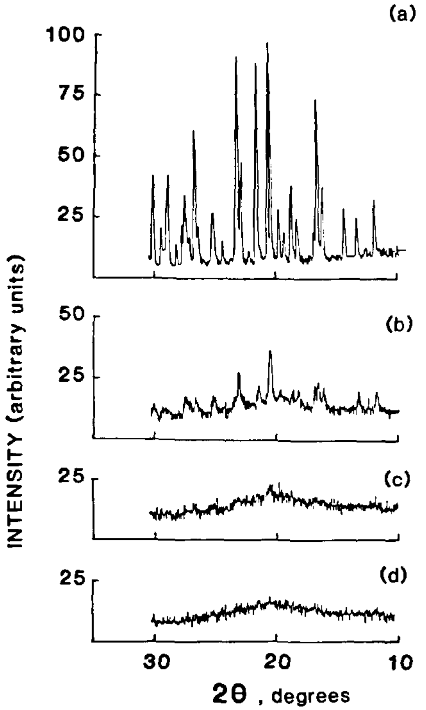

Fig. 8. Powder X-ray diffraction patterns of I (a) unground; (b), (c) and (d) ground for 15, 30 and 60 min, respectively.

TABLE 1

EFFECT OF GRINDING ON SOME PROPERTIES OF II

Grinding time (min)	Apparent solubility (molal)	Surface area ($m^2 \cdot g^{-1}$)	Density ($m^2 \cdot g^{-3}$)	Particle diameter (assuming spherical particles) (Å)	Heat of solution ($kJ \cdot mol^{-1}$)	% crystallinity by:		
						X-ray	heat of solution	density
0	1.29	5.45	1.6621	6620	+13.77	100 *	100 *	100 *
15	1.67	8.16	1.6720	4400	+5.91	72.4	61.8	68.3
30	2.41	8.05	1.6782	4380	−1.46	32.2	31.3	48.4
60	2.96	10.51	1.6822	3400	−6.09	24.8	12.2	35.6
240	ND	14.08	1.6933	2500	−9.13	0.0 *	0.0 *	0.0 *

* assumed.

ND = not determined.

grinding for 1 h increased the apparent solubility to 0.11 molal (Fig. 7). There was a progressive decrease in the intensity of the X-ray diffraction peaks with increase in grinding time, suggesting a decrease in the crystallinity of the sample (Fig. 8). When I was dehydrated to II, the apparent solubility increased dramatically from 0.07 to 1.3 molal. Grinding II resulted in further increases in apparent solubility. Fig. 7 shows that the effect of grinding on the apparent solubility of II was much more marked than on I. The apparent solubility of II ground for longer than 2 h could not be determined because the solutions were extremely unstable and precipitated very rapidly. The precipitate had a powder X-ray diffraction pattern identical with that of I, and is therefore the crystalline 3.5 hydrate. The precipitate from solutions of ground II is also the same as that obtained previously from solutions of the commercial amorphous anhydrate (Suryanarayanan and Mitchell, 1984).

The crystallinity of II decreased with duration of grinding, but Table 1 shows poor agreement between the percent crystallinity values determined using X-ray diffraction, heat of solution and density methods. The degree of crystallinity determinations were confined to unground and ground II because of the significant effect of grinding on apparent water solubility. The relationship between degree of crystallinity and apparent solubility is shown in Fig. 9.

When examined by means of a polarizing microscope, the commercial amorphous anhydrate was non-birefringent compared with II, which consisted of highly birefringent needle crystals. The size of the needle crystals decreased with grinding time, but after about 30 min grinding, the particles started to form aggregates which were readily redispersed in the mineral oil mounting liquid. The deaggregated material retained its birefringent character for up to 2 h of grinding, but after 4 h grinding, many of the particles were non-birefringent (i.e. amorphous). Although the 4-h

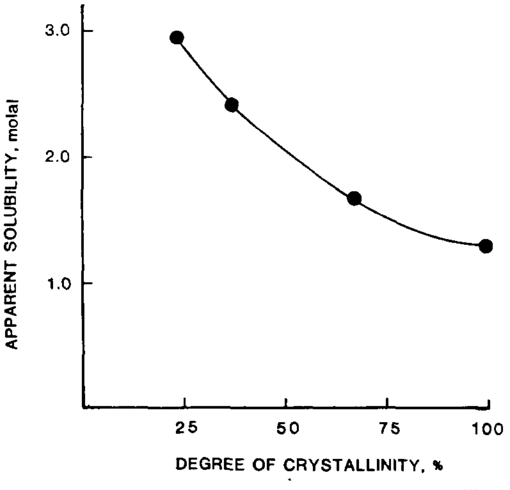

Fig. 9. Relationship between the degree of crystallinity and apparent solubility of II in water at room temperature (~ 22°C). The individual crystallinity values are averages of the values determined by X-ray, calorimetry and density for each grinding time (Table 1).

ground II was amorphous to X-rays, Fig. 2b, it is apparent that polarized light microscopy was a more sensitive indicator of crystallinity than X-ray diffraction, since some particles still showed birefringence and extinction positions. However, examination by means of the polarizing microscope gave no indication of the progressive decrease in crystallinity with grinding time.

In the pharmaceutical literature, the degree of crystallinity has usually been calculated on the assumption that the two-state model is applicable (Black and lovering, 1977; Pikal et al., 1978; Nakai et al., 1982). The X-ray crystallinity is often calculated according to the following relationship (Klug and Alexander, 1974):

$$\text{Percent crystallinity} = \frac{\mathrm{Ic}}{\mathrm{Ic} + \mathrm{Ia}} \times 100 \tag{2}$$

where Ic and Ia are respectively the crystalline and amorphous intensities of diffracted X-rays. Thus the total energy of diffracted radiation from the crystalline and amorphous components may be considered as proportional to the quantity of crystalline and amorphous phases present, respectively (Clark and Terford, 1955).

Using the calorimetric method, percent crystallinity is given by:

$$\frac{\Delta H_s - \Delta H_a}{\Delta H_c - \Delta H_a} \times 100 \tag{3}$$

where ΔH_s, ΔH_c and ΔH_a are the heats of solution to infinite dilution in any fixed solvent, of the sample, the 100% crystalline standard and the 0% crystalline (amorphous) standard, respectively. In this work, percent crystallinity by both X-ray diffraction and calorimetry was determined from standard curves obtained by mixing various proportions of the crystalline and amorphous standards (Figs. 3 and 5). Eqns. 2 and 3 are based on a two-state model for polymers, according to which small but perfect crystalline regions (crystallites) are embedded within a continuous amorphous matrix (Miller, 1966a). However, even in polymers, such a model is recognized as a gross oversimplification (Miller, 1966b), and its relevance to most pharmaceuticals is questionable because of the differences between polymers and other crystalline materials.

Density measurements of ground II were undertaken both as an alternative method of measuring the degree of crystallinity and as a method of testing the models of crystallinity. Contrary to expectations, the results in Table 1 show that the density gradually increased with grinding time, suggesting that the crystals initially have an open lattice which gradually collapses under mechanical stress. According to the two-state model illustrated in Fig. 1, the decrease in crystallinity with grinding is due to a progressive conversion of crystalline material to the amorphous state. However, dispersion of ground II in the carbon tetrachloride–ethylene dibromide mixture did not result in separation into two fractions corresponding to the crystalline and amorphous states. Instead, there was a gradual and progressive change in the density of II with increasing grinding time, which suggests the one-state model. However, as discussed above, other models of crystallinity are possible. For example, the surface of a crystal may become amorphous on grinding (Khodakov and Rebinder, 1961) and the thickness of the amorphous layer may increase with grinding time until the whole particle is amorphous. When dispersed in the suspending liquid, the solid would not separate into two fractions even though each particle contains both amorphous and crystalline phases. Hence, by itself, the suspension density method does not provide unequivocal evidence for a particular model.

Microscopical examination using polarized light showed that all particles were birefringent even after 2 h of grinding. If the surface amorphization model were correct, birefringency would be expected to disappear very quickly on grinding. It would be expected that the increasingly thick amorphous layer would cause the disappearance of birefringence after a few minutes of grinding. Hence, the formation of an amorphous layer surrounding a crystalline core does not describe the decreasing crystallinity of II as grinding continues. It is probable that grinding causes more disorder in the surface layers of a particle than in the bulk, and that the disorder progressively decreases towards the core of each particle. If the two-state model were correct, the majority of the particles should be non-birefringent after 1 h of grinding because the crystallinity value is less than 50% (Table 1).

Although the density of a solid is independent of particle size, it was important to verify that the X-ray line broadening and changes in the heat of solution values were not simply due to a decrease in particle size on grinding. From the surface area determined by krypton adsorption and the density, a hypothetical particle size was determined for each sample, assuming that all the particles were spherical in shape and of uniform size (Table 1). The effect of particle size on X-ray line breadth usually becomes apparent only when the crystal size is below 1000 Å (Cullity, 1978). Since, after 4 h grinding, the hypothetical particle diameter of II was about 2500 Å, it can be concluded that the X-ray line broadening is mainly a consequence of distortion of the crystal lattice rather than particle size reduction.

Brunauer et al. (1956a) observed that a 26-fold increase in surface area of calcium oxide (from 0.3 to 7.8 m^2 g^{-1}) produced a decrease in total enthalpy of 0.56 $kJ \cdot mol^{-1}$ (from -198.19 $kJ \cdot mol^{-1}$ to -198.75 $kJ \cdot mol^{-1}$). Further work by Brunauer et al. (1956b, 1959) confirmed that very large increases in surface area produced only small changes in enthalpy. The results in Table 1 show that crystalline II with an endothermic heat of solution was rendered X-ray amorphous on grinding with an accompanying exothermic heat of solution, i.e. a very large enthalpy change for a small increase in surface area. Dialer and Kuessner (1973) observed a similar effect on milling crystalline sucrose, which resulted in its transformation into a glass-like material. The accompanying change in enthalpy could not be accounted for by the increase in surface area alone. According to Calvet and Prat (1963), crystal dissolution is preceded by the exothermic adsorption of solvent molecules on the solute surface, followed by an endothermic breakup of the crystal lattice. Amorphous solids are characterized by the absence of long range order in their crystal lattice. When they dissolve in a solvent, less energy is required to break up the lattice and the overall heat of solution generally becomes exothermic. Thus the change from the endothermic to exothermic heat of solution on grinding II is attributed to a progressive change from an ordered lattice to a highly disordered lattice containing excess free energy.

The increase in apparent solubility of II on grinding is also attributed to the increase in lattice disorder. Because of the method by which the apparent solubility was determined, the experimental value will depend on the dissolution rate of II as well as equilibrium solubility of I. Burt and Mitchell (1981) showed that differences in dislocation density in potassium perchlorate crystals, induced by changes in crystal growth rate, caused a significant increase in the dissolution rate constant. Friesen et al. (1981) increased the number of dislocations from an initial value of 3.5×10^3 cm^{-2} up to approximately 2.5×10^5 cm^{-2} by mechanically stressing single crystals of potassium perchlorate, and produced a 40% increase in the dissolution rate constant. The much greater stress of grinding can be expected to introduce much larger numbers of dislocations and other defects into a crystal, and it is suggested that the resulting decrease in crystallinity is responsible for the increase in apparent solubility of II.

In addition to the creation of lattice disorder, grinding may increase apparent solubility both as a result of particle size reduction and by exposing more reactive crystal faces to the dissolution medium. The effect of particle size reduction on solubility can be calculated from the Ostwald-Freundlich equation (Florence and Attwood, 1982), but the 2.3-fold increase in apparent solubility of II after grinding for 60 min is far greater than could be accounted for by the 2.0-fold reduction in hypothetical particle size [2]. Since II occurred as a needle habit, the surfaces created on fracture may be more reactive than surfaces parallel to the needle axis. The effects of crystal anisotropy and habit modification in nickel sulfate hexahydrate crystals have been studied by Burt and Mitchell (1979, 1980), and although significant effects on dissolution rate constants were observed, the contribution of dissolution anisotropy towards the overall increase in apparent solubility found in this work is likely to be minimal compared with the effect due to increased disorder.

A major difficulty with calculating percent crystallinity is the selection of appropriate crystalline and amorphous standards. The perfect crystal does not exist, and the material used as the 100% crystalline standard (unground II) will contain numerous defects (reduced crystallinity) as a result of its preparation from I by dehydration. Similarly, II ground for 4 h, selected as the 0% crystalline standard because it was X-ray amorphous, does not represent a true amorphous state since some particles still showed birefringence when examined by means of polarized light microscopy [3]. Degree of crystallinity values obtained using one set of standards and a particular experimental method are not likely to agree with values obtained using

---

[2] Assuming arbitrary surface energy values ranging from 1×10^{-6} J·cm^{-2} to 5×10^{-5} J·cm^{-2} for II, the calculated solubility ratio at 25°C of the 1 h ground (hypothetical particle diameter 3400 Å) to unground sample (hypothetical particle diameter 6620 Å) would range from 1.01 : 1.00 to 1.41 : 1.00.

[3] Amorphous anhydrous calcium gluceptate (Pfanstiehl Lot 12953-D) could have been used as the 0% crystalline standard, but we had only a very small quantity of this material and it is no longer commercially available.

either other standards or another method, and Otsuka and Kaneniwa (1983) have shown that even using two X-ray diffraction methods resulted in different values for the crystallinity of cephalexin. Hence, too much importance should not be attached to the numerical values of percent crystallinities. Nevertheless, they provide a useful indication of the state of order of a solid and can be correlated with other properties of the solid state which are profoundly influenced by changes in the state of order.

Conclusions

(1) Dehydrating calcium gluceptate hydrate produced a dramatic increase in apparent water solubility.

(2) Grinding dehydrated calcium gluceptate caused further increases in apparent water solubility.

(3) Grinding reduced particle size and decreased the degree of crystallinity, but only the latter was important with respect to the increases in apparent solubility.

(4) The degree of crystallinity was assessed by X-ray, heat of solution and density measurements, but the values were not in good agreement.

(5) It is suggested that the decrease in crystallinity with grinding is due to an increase in lattice disorder throughout the entire sample (one-state model) rather than to a progressive increase in the proportion of amorphous material in a crystalline-amorphous mixture (two-state model).

Acknowledgements

This work was presented at the 131st Annual Meeting of the American Pharmaceutical Association in Montreal, Quebec, Canada, May 5–10, 1984. We thank Mr. R.G. Butters of the Department of Metallurgical Engineering for his help in the X-ray work, and Ms. Catherine Hannah for technical assistance. This investigation was supported by a research grant from the Medical Research Council of Canada.

References

Alexander, L.E., X-Ray Diffraction Methods in Polymer Science, Wiley, New York, U.S.A. 1969, p. 176.

Black, D.B. and Lovering, E.G., Estimation of the degree of crystallinity in digoxin by X-ray and infrared methods. J. Pharm. Pharmacol., 29 (1977) 684–687.

Brunauer, S., Kantro, D.L. and Weise, C.H., The surface energies of calcium oxide and calcium hydroxide. Can. J. Chem., 34 (1956a) 729–742.

Brunauer, S., Kantro, D.L. and Weise, C.H., The surface energies of amorphous silica and hydrous amorphous silica. Can. J. Chem., 34 (1956b) 1483–1496.

Brunauer, S., Kantro, D.L. and Weise, C.H., The surface energy of tobermorite. Can. J. Chem., 37 (1959) 714–724.

Burt, H.M. and Mitchell, A.G., Dissolution anisotropy in nickel sulfate α hexahydrate crystals. Int. J. Pharm., 3 (1979) 261–274.

Burt, H.M. and Mitchell, A.G., Effect of habit modification on dissolution rate. Int. J. Pharm., 5 (1980) 239–251.

Burt, H.M. and Mitchell, A.G., Crystal defects and dissolution. Int. J. Pharm., 9 (1981) 137–152.

Calvet, E. and Prat, E., Recent Progress in Microcalorimetry, Pergamon, Oxford, U.K., 1963, p. 85.

Clark, G.L. and Terford, H.C., Quantitative X-ray determination of amorphous phase in wood pulps as related to physical and chemical properties. Anal. Chem., 27 (1955) 888–895.

Cullity, B.D., Elements of X-ray Diffraction, 2nd edn., Addison-Wesley Press, Reading, U.S.A., 1978, pp. 284 and 285.

Dialer, K. and Kuessner, K., Surface area generation and energy uptake during oscillating milling of refined sugar. Influence of bonding on extreme size reduction. Kolloid-Z. Z. Polym., 251 (1973) 710–715; through Soc. Chem. Abstr., 80 (1974) 122647p.

Estermann, I., Leivo, W.J. and Stern, O., Change in density of potassium chloride crystals upon irradiation with X-rays. Phys. Rev., 75 (1949) 627–633.

Florence, A.T. and Attwood, D., Physicochemical Principles of Pharmacy, Chapman and Hall, New York, U.S.A., 1982, p. 139.

Friesen, M., Burt, H.M. and Mitchell, A.G., Crystal dislocations and dissolution. J. Pharm. Pharmacol., 33 (1981) 22P.

Huttenrauch, R., Molecular galenics as the basis of modern drug formation. Acta Pharm. Tech., Suppl. 6 (1978) 55–127.

Imaizumi, H., Nambu, N. and Nagai, T., Stability and several physical properties of amorphous and crystalline forms of indomethacin. Chem. Pharm. Bull., 28 (1980) 2565–2569.

Johnston, H.L. and Hutchison, C.A., Efficiency of the electrolytic separation of lithium isotopes. J. Chem. Phys., 8 (1940) 869–877.

Khodakov, G.S. and Rebinder, P.A., Mechanism of quartz comminution in surface active media. Kolloid Zhur., 23 (1961) 482–490; through Soc. Chem. Abstr., 57 (1962) 2016h.

Klug, H.P. and Alexander, L.E., X-ray Diffraction Procedures for Polycrystalline and Amorphous Materials, 2nd ed., Wiley, New York, U.S.A., 1974, p. 560.

Lange's Handbook of Chemistry, J.A. Dean (Ed.), 12th edn., McGraw-Hill, New York, U.S.A., 1979, pp. 10–103.

Lowell, S., Continuous flow krypton adsorption for low surface area measurements. Anal. Chem., 45 (1973) 1576–1577.

Merck Index, Ed. M. Windholz, 10th edn., Merck and Co., Rahway, U.S.A., 1983, (a) p. 853, (b) p. 869.

Miller, R.L. in Encyclopedia of Polymer Science and Technology, Vol. 4, Wiley, New York, U.S.A. 1966, (a) p. 451, (b) p. 453.

Nakai, Y., Fukuoka, E., Nakajima, S. and Hasegawa, J., Crystallinity and physical characteristics of microcrystalline cellulose. Chem. Pharm. Bull., 25 (1977) 96–101.

Nakai, Y., Fukuoka, E,. Nakajima, S. and Morita, M., Physicochemical properties of crystalline lactose. Estimation of the degree of crystallinity and the disorder parameter by an X-ray diffraction method. Chem. Pharm. Bull., 30 (1982) 1811–1818.

Otsuka, M. and Kaneniwa, N., Effect of grinding on the degree of crystallinity of cephalexin powder. Chem. Pharm. Bull., 31 (1983) 4489–4495.

Pikal, M.J., Lukes, A.L., Lang, J.E. and Gaines, K., Quantitative crystallinity determinations for β-lactam antibiotics by solution calorimetry: correlations with stability. J. Pharm. Sci., 67 (1978) 767–773.

Suryanarayanan, R. and Mitchell, A.G., The stability of calcium glucoheptonate solutions. J. Pharm. Pharmacol., 33 (1981) 12P.

Suryanarayanan, R. and Mitchell, A.G., Precipitation of calcium gluceptate from aqueous solutions. J. Pharm. Sci., 73 (1984) 78–82.

The United States Pharmacopeia, 20th revision, United States Pharmacopeial Convention, Rockville, U.S.A., 1980, (a) p. 990, (b) p. 107.

Vaughan, W.H., Leivo, W.J. and Smoluchowski, R., Density and hardness changes produced by plastic deformation in potassium chloride crystals. Phys. Rev., 110 (1958) 652–657.

York, P., Solid-state properties of powders in the formulation and processing of solid dosage forms. Int. J. Pharm., 14 (1983) 1–28.