STUDIES ON THE CRYSTALLINITY AND PHASE TRANSITIONS OF CALCIUM GLUCEPTATE

by

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ABSTRACT

Calcium gluceptate (calcium α-glucoheptonate, $CaC_{14}H_{26}O_{16}$) occurs as a crystalline hydrate (I) containing 3 1/2 molecules of water of crystallization per atom of calcium or as an amorphous anhydrate(III). Calcium gluceptate was synthesised commercially as III until 1980 but since then only I has been commercially available. The maximum aqueous solubility of III at room temperature was found to be > 2 molal while the equilibrium solubility of I at 25.5°C was 0.06 molal (≃3.3% w/v). A crystalline anhydrate (II) which had an apparent water solubility of 1.3 molal was prepared from I by dehydration. The United States Pharmacopeia (USP) injection is an aqueous solution containing 20.8 to 23.3% w/v $CaC_{14}H_{26}O_{16}$ which is greater than the equilibrium solubility. Solutions prepared using II or III were supersaturated with respect to I and crystallized on storage. Pharmaceutically stable solutions could be prepared from II by autoclaving the solutions at 121°C for 20 minutes immediately after preparation which presumably destroyed seed crystals of I. When stored at relative humidities (RH) greater than 66% at 25°C, II was converted into I and the reverse process occurred at 0% RH. Above 0% and below 66% RH neither I nor II underwent a phase transition during one year of storage. The co-existence of I and II over a range of RH would be contrary to the phase rule. It is suggested that at RH less than 66%, the adsorption of a small amount of atmospheric water vapor inhibits the II to I

transition probably due to the formation of a surface layer of I which limits further diffusion of water. In addition to the phase transformations in aqueous solution and the dehydration and rehydration reactions, the effects of freeze drying and grinding on the interconvertibility of I, II and III were studied. On grinding 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 were attributed to decreases in crystallinity, since surface area measurements showed that they could not be due to particle size reduction. Density is independent of particle size, and values obtained for II using a liquid suspension method changed progressively with grinding. The x-ray diffraction, calorimetric and density measurements were used both to quantify the degree of crystallinity of II and to test various models of crystallinity. According to the USP, solids are 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 100% (perfect crystal) and 0% (amorphous) depending on the state of order/disorder in the lattice (one-state model). It was concluded that grinding decreases the crystallinity of II by increasing lattice disorder according to the one-state model.

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INTRODUCTION

A. SOLIDS

Solids as defined by the ability to transmit shear waves, or by a minimum viscosity of 10^{13} N m^{-2} s (10^{14} poise), may be either crystalline or non-crystalline (Roy, 1970). A solid is considered crystalline, if there is long range periodicity of the arrangement of the constituent atoms, ions or molecules to a general minimum of 3-5 nm(30-50 Å). Another arbitrary designation is that crystalline materials are characterized by three-dimensional periodicity over distances of six or more unit cells (Klug and Alexander, 1974a). All remaining solids may be grouped as non-crystalline solids. There is no sharp boundary between crystalline and non-crystalline solids and the relationship between the two is given in Fig. 1. Non-crystalline solids obtained from crystalline solids may also be called "amorphous solids", while a non-crystalline solid produced from a liquid is referred to as "glass" (Fig. 1). In the non-crystalline solids, free energy in excess over the stable crystalline solid is included into the system and this may be achieved by a sudden change of a thermodynamic variable like temperature or pressure.

B. CONCEPTS OF CRYSTALLINITY

The crystalline state, characterized by the perfectly ordered crystal lattice and the non-crystalline (amorphous)

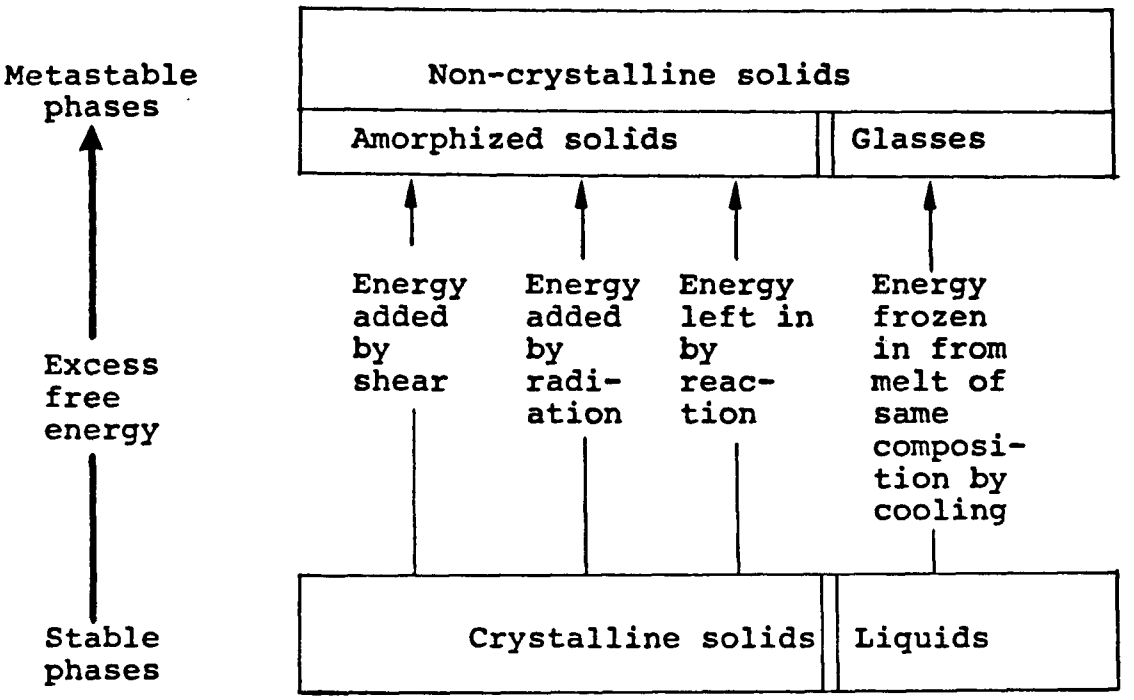


Fig. 1 The relationship between crystalline and non-crystalline solid phases. The relationship of liquids to glasses is also shown (Roy, 1970).

state represented by a disordered lattice, represent two extremes of lattice order and intermediate states are possible. The term degree of crystallinity is useful in attempts to quantitate these intermediate states. There are broadly two concepts of crystallinity of solids.

1. Two-state model of crystallinity

The following discussion is based on extensive investigations of polymer crystallinity which were reviewed by Miller (1966a). Many polymers exhibit properties associated with both crystalline materials (e.g., diffraction of x-rays, evolution of latent heat on cooling from the melt) and non-crystalline materials (e.g., diffuse x-ray scattering). This behavior can be explained by the two-state (or fringed-micelle) model according to which polymeric materials consist of small but perfect crystalline regions (crystallites) which are embedded within a continuous amorphous matrix. It is believed that a given molecule of polymer passes from one crystallite to the next through a diffuse, amorphous region at the crystallite ends. The properties of each crystalline and amorphous phase are assumed to be independent of the presence and amount of the other. An observed property is taken as the sum of extensive properties of the two phases, i.e., the properties of "crystalline" and "amorphous" phases are additive. For example, the x-ray diffraction pattern would be considered as the superposition of diffraction from the crystalline regions and of scattering

from the amorphous regions. The percent crystallinity, P, is calculated from:

$$P = \frac{p - p_a^o}{p_c^o - p_a^o} \times 100 \qquad (1)$$

where p is a specific property (e.g., specific volume) of the sample under investigation, and p_a^o and p_c^o are the specific properties of the completely amorphous and completely crystalline samples respectively.

According to the USP (USP XX, 1980a), solids are either crystalline, non-crystalline or a mixture of the two. The degree of crystallinity depends on the fraction of crystalline material in a mixture of the amorphous and crystalline states. Clearly, the USP subscribes to the two-state model of crystallinity and the decreasing crystallinity according to this model is shown schematically in Fig. 2.

2. One-state Model

As the two-state model was refined, the division of a sample into two idealized regions, crystalline and amorphous, was realized to be unrealistic and arbitrary. Thus the oversimplified nature of the two-state model became apparent even though the model was capable of explaining some properties of polymers. Moreover, the crystallinity values of the same sample obtained using different methods often failed to agree and this was attributed to the departure of the actual structure of the polymer from that of the idealized

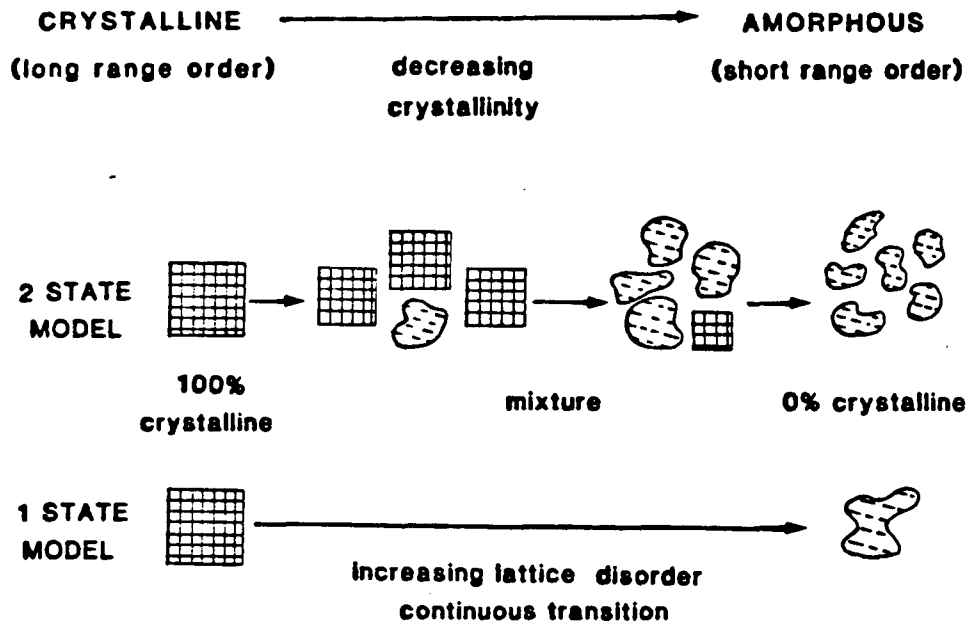


Fig. 2 Schematic representation of the two models of crystallinity. Squares represent the lattices of perfect crystals, while irregular shapes represent the amorphous state; no assumptions are implied regarding the structure of the latter.

two-state model (Miller, 1966b). Different experimental techniques weigh the spectrum of order present differently and Miller (1966b) concluded that agreement among crystallinity determinations by different techniques was fortuitous. It was suggested therefore that the word "crystallinity" should always be modified by an experimental adjective, e.g., x-ray crystallinity, density crystallinity, etc. Moreover, the two-state model was developed using polymeric materials. The application of such a model to most pharmaceuticals is questionable because of the differences between polymers and other crystalline materials.

An alternative concept has emerged, according to which, the degree of crystallinity has a value located on a continuous scale which varies between 100% and 0% depending on the state of order/disorder in the lattice (Miller, 1966a; Huttenrauch, 1978). The 100% crystalline material is considered to have a perfectly ordered lattice while the 0% crystalline (or non-crystalline or amorphous) state is characterized by a completely disordered lattice. The transition from the 100% to 0% crystallinity is brought about by increasing lattice disorder. Since there is no sharp distinction between the crystalline and amorphous states, this model is referred to as a one-state model.

Disorder in a crystal lattice could be produced by imperfections (defects) in the lattice and an increasing concentration of imperfections progressively increases the

disorder causing a decrease in the degree of crystallinity. Crystal defects are broadly classified into:

1. Zero-dimensional defects. These are localized imperfections and are referred to as point defects. Different types of point defects are produced by (Carstensen, 1973b):
   a. vacant lattice sites (vacancies)
   b. atoms, molecules or ions found in metastable interstitial positions (interstitials). Foreign species may also occupy interstitial positions.
2. One dimensional defects. The most important defects in this category are dislocations. They are of two possible types - edge dislocations and screw dislocations. An edge dislocation may be thought of as the insertion of an extra plane of atoms terminating along the line of dislocation (Vickery, 1983). In a screw dislocation, the atomic planes are joined together in such a way as to form a spiral staircase, winding round the line of dislocation.
3. Two dimensional defects. The two types of crystal defects in this category of potential pharmaceutical significance are:
   a. Grain boundaries. In many solids, each crystal is composed of numerous crystallites. Each of the crystallites is misoriented with its neighbor to a greater or lesser extent and the grain boundary is a transition zone between crystallites (Friedel,

1964).

b. Stacking faults. These are planes across which the regular stacking sequence of the crystal structure is altered (Holt, 1980).

The two models schematically shown in Fig. 2 represent, in a highly simplified way, the complex transition from the crystalline to the amorphous state. It is recognized that other models or combinations of models are possible.

A direct correlation between the defect content of crystals and their degree of crystallinity has not yet been established. This is because there are no known experimental techniques that can simultaneously quantitate different types of crystal imperfections. Moreover, the presence of crystal imperfections alone is unlikely to make a material completely non-crystalline. As discussed earlier, the amorphous or non-crystalline state is characterized by the absence of long range periodicity and the imperfections discussed above, even if present in overwhelming concentration, are probably incapable of causing complete lattice disorder. There is also a limit to the maximum attainable concentration of certain types of imperfections. For example, the maximum number of dislocations possible is around 10^{12} cm^{-2} (Vickery, 1983).

C. METHODS OF QUANTITATING CRYSTALLINITY

In order to fully characterize the solid phase, precise methods for determining the degree of crystallinity are

desired. Some of the experimental methods for quantitation of crystallinity are described below.

1. Powder x-ray Diffraction

Powder x-ray diffraction is the method most widely used to determine the degree of crystallinity of pharmaceuticals (e.g., Black and Lovering, 1977; Nakai *et al.*, 1977, 1982). The total energy of the diffracted radiation from the crystalline and amorphous components may be considered as proportional to the quantity of crystalline and amorphous phases respectively (Clark and Terford, 1955). The percent crystallinity, P_x, from powder x-ray diffraction studies can therefore be calculated according to the following relationship (Klug and Alexander, 1974b):

$$P_x = \frac{I_c}{I_c + I_a} \times 100 \qquad (2)$$

where I_c and I_a are respectively the crystalline and amorphous intensities of diffracted x-rays. In the measurement of diffraction line intensity, integrated intensity rather than the maximum intensity must be measured because of possible variations in line shape due to variations in microstrain (disorder) and particle size (Cullity, 1978a). Chaklader (1963) found that even the integrated intensity values of large particles (>43 μm diameter) of quartz were highly variable but smaller particles exhibited reproducible peak intensities. In the quantitation of crystallinity from integration of peak areas, the methods of Ruland (Ruland,

1961) and of Hermans (Hermans and Weidinger, 1948) have been reported in the pharmaceutical literature (Nakai *et al.*, 1977, 1982; Otsuka and Kaneniwa, 1983; Morita and Hirota, 1982). The main problem in the use of integrated peak intensity is that the separation of amorphous scattering from the total diffraction pattern is, at best, arbitrary (Alexander, 1969a). If the shape of the diffraction lines are not affected by variations in disorder and particle size, then the maximum intensities rather than integrated intensities may be used as a measure of diffraction line intensity. The quartz content of dusts have been determined with satisfactory accuracy by simply measuring maximum intensities (Cullity, 1978b) and a similar method may be used to determine the degree of crystallinity, provided the experimental sample satisfies the above stated condition. Measurement of absolute intensity would, however, require correction for background scattering. This requirement can be overcome by the use of an internal standard. An ideal internal standard would, (a) have a diffraction pattern which does not interfere with that of the material being analyzed, (b) be of high crystal symmetry so that strong but few diffraction peaks are produced, (c) have a density close to that of the sample under investigation so that homogenity in mixing is maintained and (d) be chemically stable (Shell, 1963).

Two problems associated with the powder x-ray diffraction method are the effects of sample orientation (preferred orientation) and particle size on x-ray intensity. Reduction

of particle size by prolonged grinding is the single most effective means for minimizing preferred orientation errors (Klug and Alexander, 1974c). Since grinding reduces the crystallinity of many materials (e.g., Motooka *et al.*, 1969; Florence *et al.*, 1974; Lee and Hersey, 1977; Otsuka and Kaneniwa, 1983), it cannot be used in crystallinity determinations. Moreover, decreased particle size can cause a broadening of x-ray lines and this effect usually becomes apparent when the particle size is below 100 nm (1000 Å). The Scherrer formula relates the x-ray line breadth, β (this is the angular width usually measured in radians) to the size, t, of a crystal (Cullity, 1978c):

$$t = \frac{0.9\lambda}{B\cos\theta_B} \qquad (3)$$

where λ is the wavelength of x-rays used and θ_B is the angle of peak diffraction. Carbon black has been used as a dispersing agent to prevent both agglomeration and orientation of penicillin crystals but this required prior grinding of the sample with carbon black (Christ *et al.*, 1948). Chaklader (1963) used the following procedure to minimize preferred orientation and nonuniform packing of particles. The powder was packed into the rectangular cavity of the sample holder, the surface levelled with a glass slide and x-ray counts obtained. The surface of the sample was then disturbed and relevelled and x-ray counts again obtained. This procedure was repeated several times and the mean count value was determined. Otsuka and Kaneniwa (1983) used lithium fluoride

as an x-ray internal standard but also believed that it acted as a diluent and resisted preferred orientation of sample crystals.

2. Calorimetry

2.1 Solution calorimetry

The use of solution calorimetry is based on the observation that, for many solids, the energy of the amorphous form is significantly higher than the energy of the crystalline form (Pikal *et al.*, 1978). The percent crystallinity from solution calorimetry, P_s, is defined as:

$$P_s = \frac{\Delta H_s - \Delta H_a}{\Delta H_c - \Delta H_a} \times 100 \qquad (4)$$

where ΔH_s, ΔH_a and ΔH_c are the heats of solution to infinite dilution (in any fixed solvent) of the sample, the 0% crystalline (amorphous) standard, and the 100% crystalline standard respectively. If the energy difference between the amorphous and crystalline states is large, calorimetric crystallinities are potentially more precise than crystallinity data derived from x-ray diffraction (Pikal *et al.*, 1978).

The data in Table I was obtained by a variety of methods but shows that the difference in heats of solution between the amorphous and crystalline forms of a compound can be quite large thus making solution calorimetry a potentially useful technique.

Table I Heats of solution of some compounds in both crystalline and amorphous states.

Compound	Physical form	Heat of solution ($kJ\ mol^{-1}$)	Reference
Sulfathiazole[a]	crystalline, form I	+38.7	Simonelli et al., 1976
	x-ray amorphous, co-precipitated	+ 3.37	
Zinc sulfate monohydrate[b]	crystalline	-44.9	Frost et al., 1951
	x-ray amorphous, prepared by vacuum dehydration of heptahydrate	-76.7	
Copper sulfate monohydrate[b]	crystalline	-42.3	Frost et al., 1951
	x-ray amorphous, prepared by vacuum dehydration of pentahydrate	-72.3	
Cefamandole nafate[c]	crystalline, Y-form	+8.03	Pikal et al., 1978
	x-ray amorphous, freeze dried	-18.4	
Cephalothin sodium[c]	crystalline	+7.95	Pikal et al., 1978
	x-ray amorphous, freeze dried	-17.2	
Penicillin G potassium[c]	crystalline	- 1.34	Pikal et al., 1978
	x-ray amorphous, freeze dried	-22.6	

[a]Calculated from van't Hoff plots.

[b]The heats of solution of several crystalline zinc sulfate samples containing varying percentages of water of crystallization were determined, and a linear relationship between heat of solution and water content was established. The heat of solution of crystalline zinc sulfate monohydrate was obtained from this relationship. A somewhat similar procedure was used for determining the heats of solution of amorphous zinc sulfate monohydrate, and crystalline and amorphous copper sulfate monohydrate.

[c]Determined directly by solution calorimetry.

The use of Eq. (4) requires the determination of the differential heat of solution which is impractical to measure directly (Daniels and Alberty, 1967). The differential heat of solution is almost constant in very dilute solutions and under such conditions the differential and integral heats of solution are essentially equal (Glasstone and Lewis, 1982a). Hence, integral heat of solution values can be used to calculate percent crystallinity, provided the solutions are very dilute.

2.2 Fusion calorimetry

The percent crystallinity from heat of fusion, P_f, can be calculated according to the following relationship (Ke, 1966):

$$P_f = \frac{\text{heat of fusion of experimental sample}}{\text{heat of fusion of 100\% crystalline sample}} \cdot x\ 100 \quad (5)$$

Noncrystalline (amorphous) compounds are characterized by the absence of a sharp melting endotherm. An advantage of this method is the possibility of quantitating crystallinity without an amorphous reference standard and it has been used in the evaluation of crystallinity of polymers. However, the compound must not decompose before or during melting and therefore thermolabile compounds cannot be investigated.

3. Density

Density measurements can 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. The free volume of salt-type substances in the liquid state (which is a disturbed and thermally excited state) is about 29% of the total volume, but in solids it is only about 26% (Huttenrauch, 1978). Since this alteration proceeds in a linear manner, there is a linear relationship between density and degree of order. If the density of the perfectly crystalline (100% crystalline) solid, ρ_c, and the density of the same material in the amorphous (0% crystalline) state, ρ_a, are taken as the two limits, the percent crystallinity of the sample under investigation, P_d, can be deduced from the following relationship:

$$P_d = \frac{\rho - \rho_a}{\rho_c - \rho_a} \times 100 \qquad (6)$$

where ρ is the density of the sample under investigation. The density of a perfect crystal ρ_c can be calculated from (Alexander, 1969b):

$$\rho_c = \frac{MZ}{AV} \qquad (7)$$

where M is the molecular weight, Z is the number of molecules per unit cell, A is the Avogadro number and V is the volume of the unit cell. The unit cell parameters are usually determined from single crystal x-ray studies. The density of the amorphous state can be approximated from group contributions to molar volume (Grant, 1983). This method is however

restricted to those compounds containing functional groups of known molar volume values (Rheineck and Lin, 1968; Exner, 1967).

Several techniques are available for the determination of the density of solids (Bauer and Lewin, 1972), but the suspension density method is unique because it is possible not only 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 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 to be 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 must be accompanied by gradual, progressive changes in density.

The suspension density method, in a highly refined form, was developed and used by Hutchison and Johnston (1940) for the precise determination of the relative densities of lithium fluoride samples within an error limit of about $\pm 5 \times 10^{-6}$ g cm^{-3} and the absolute densities to within an error about 10 times larger. Vaughan *et al*. (1958) studied the density changes which accompany plastic deformation on compressing potassium chloride crystals. Using the suspension density technique, they were able to quantitate a density decrease of 18.6 x 10^{-6} g cm^{-3} per percent deformation. In the pharmaceutical literature, Huttenrauch and Keiner (1976a) suggested the use of the suspension density method for determining the degree of crystallinity (degree of order). They used carbon tetrachloride as the suspending liquid for microcrystalline cellulose since its density varies from 1.584 g cm^{-3} at 20°C (Lange's Handbook of Chemistry, 1979b) to 1.496 g cm^{-3} at 70°C (Timmermans, 1950) compared with the change in density from 1.588 g cm^{-3} for crystalline cellulose to 1.482 g cm^{-3} for amorphous cellulose. The crystallinity of cellulose was progressively decreased by grinding. The changes in crystallinity of lactose with grinding were also investigated by similar methods (Huttenrauch and Keiner, 1976b).

4. Infrared (IR) Spectroscopy

There seems to be no predictable relationship between the degree of crystallinity of a compound and its IR absorption behavior. According to Kossler (1967), some IR absorption

bands of polymers may appear only when the material exists in a crystalline state. These can be identified as truly crystalline bands if: (a) x-ray diffraction data prove the material is crystalline, (b) the band disappears on melting and (c) other IR studies (e.g., solid solutions in isomorphous matrices) show that the band depends on the existence of a crystal lattice (Zerbi and Ciampelli, 1964). Yariv and Mendelovici (1979) found that poorly crystalline hematite had 3 absorption bands at 308, 445 and 530 cm^{-1} which were assigned as oxygen displacements.The oxygen displacement absorption bands in well crystallized hematite shifted to higher frequencies of 333, 468 and 543 cm^{-1}. Sharp IR bands were observed with anhydrous ampicillin while ampicillin monohydrate exhibited diffuse bands which were indicative of a low degree of order (Grant and Alburn, 1965). Similar results have been obtained with calcium gluceptate. X-ray amorphous, anhydrous, calcium gluceptate had a few large and poorly defined absorption bands while the crystalline calcium gluceptate hydrate (3 1/2 molecules of water of crystallization) had sharp IR bands (Muller *et al.*, 1979; Suryanarayanan and Mitchell, 1984). Crystalline digoxin was characterized by peaks at 1775 and 3095 cm^{-1} which were absent in amorphous digoxin and the intensity of the above peaks were used to quantitate the degree of crystallinity of digoxin (Black and Lovering, 1977). Otsuka and Kaneniwa (1983) determined the degree of crystallinity of ground cephalexin by a somewhat similar method. In all the foregoing studies, the

effect of changes in crystallinity on the IR patterns have been presented. However, the relationship between the state of lattice order/disorder in a compound and its IR behavior has not been discussed mechanistically.

Freshly prepared aluminum hydroxide gel which was x-ray amorphous was subjected to IR studies by Nail *et al.* (1975). There was a broad absorption band in the 2900 - 3700 cm^{-1} region which indicated hydroxyl groups in many environments thus confirming the highly disordered nature of the fresh gel. As the gel aged, peaks appeared at 3520 and 3740 cm^{-1} with a shoulder at 3612 cm^{-1}. In the O-H deformation region, the fresh gel showed a broad peak at 900 cm^{-1} and as the gel aged a shoulder developed at 1020 cm^{-1} which eventually became a well resolved peak. The powder x-ray diffraction pattern, also underwent a similar change with the gradual appearance of peaks with aging. However, it was concluded that IR spectroscopy was more sensitive to changes in crystallinity because with IR, crystallization was evident after ≃42 days, whereas with x-ray diffraction, crystallization was evident only after ≃70 days.

In cellulose, the hydroxyl groups in the amorphous region were rapidly converted to deuteroxyl groups whereas the deuteration was very slow in the crystalline region (Nakai *et al.*, 1977). The comparison of absorption band due to hydroxyl and deuteroxyl groups in samples of cellulose ground for different periods of time was used to determine their degree

of crystallinity.

5. Other Methods

Several other techniques for quantitating crystallinity in polymers include nuclear magnetic resonance spectroscopy and relating the mechanical properties of polymers to their crystallinity (Miller, 1966c). These techniques may be of limited use in assessing crystallinity of pharmaceutical compounds.

Polarized light microscopy is widely used to assess qualitatively whether or not pharmaceutical solids are crystalline. Crystalline materials (except those belonging to the cubic crystal system) are optically anisotropic and exhibit birefringence when placed and rotated between crossed Nicol prisms (Bunn, 1946; USP XX, 1980b). On the other hand, amorphous materials being optically isotropic have only one refractive index value and are not visible between crossed Nicol prisms.

Crystalline cephalothin sodium did not undergo detectable solid-state decomposition at 50°C, but its x-ray amorphous counterpart did, resulting in the following equation which Pikal *et al.*, (1978) used to evaluate the percent crystallinity, P_t, from stability:

$$P_t = 100\left(1 - \frac{k_s}{k_a}\right) \qquad (8)$$

where k_s and k_a are the first-order decomposition rate

constants for the sample and amorphous standard respectively. This method is useful for determining the percent crystallinity of samples in those situations where the 100% crystalline standard is not available.

Since the amount of moisture adsorbed was linearly related to the degree of crystallinity of both cephalothin sodium (Pikal *et al.*, 1978) and indomethacin (Imaizumi *et al.*, 1980), this could be used as a technique to evaluate crystallinity. A similar quantitative relationship between the degree of crystallinity of cellulose and its adsorption of gentian violet was found by Huttenrauch and Keiner (1975).

The density of dislocations (line defects) provides some insight into the state of order of large 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, (iii) the method is restricted to quantitating dislocations; other types of crystal imperfections such as point defects are not included and (iv) the quantitation of dislocations is restricted to the cleaved surfaces. In addition to the above

limitations, a correlation between dislocation density and degree of crystallinity has not yet been established (see section B.2 in Introduction).

6. Identification of models of crystallinity - limitations of the methods

Most of the methods discussed above measure some change in the property of the entire sample due to changes in crystallinity. The suspension density method does not provide any direct information as to the state of lattice order of the particles but the density of the individual particles does indirectly indicate their state of lattice order. When examined microscopically using polarized light, the particles are either birefringent (crystalline) or non-birefringent (amorphous) and therefore quantitative information about intermediate states of lattice order is not obtained. Increasing lattice disorder as well as decreasing particle size can cause x-ray line broadening. None of the other techniques provide insight into the state of lattice order/disorder of the individual particles which constitute the sample. For example, a decreased enthalpy of fusion and a decreased enthalpy of solution suggest a decrease in crystallinity but give no indication of the state of order of the individual particles in the sample under investigation. Therefore, these techniques are incapable of distinguishing between the two models of crystallinity (one-state model and two-state model).

7. Comparison of quantitative crystallinity values obtained by different methods

Few workers have determined and compared the crystallinity of the same samples by different methods. Table II lists some of these studies in which the crystallinity of cellulose and cephalexin was altered by grinding and cephalothin sodium samples of varying crystallinity were prepared by freeze drying and spray drying. A comparison of the percent crystallinity values shows that the values are often in poor agreement. According to Pikal *et al*. (1978), if a partially crystalline sample were simply a mixture of the amorphous and crystalline states (two-state model), all valid measures of crystallinity would give identical results. The lack of quantitative agreement of crystallinities obtained by different methods was attributed mainly to the failure of the two-state model.

D. METHODS OF DECREASING THE DEGREE OF CRYSTALLINITY OF SOLIDS

Fig. 1 illustrates the possible ways of providing excess free energy to crystalline solids so as to convert them to their corresponding amorphous forms. Amorphous pharmaceuticals have been prepared by grinding their crystalline counterparts or from solutions by freeze drying, spray drying or precipitation (Table IV). Though all of these methods may be used to obtain samples of varying crystallinity, grinding is a convenient method because of the ease with which the degree of

Table II Comparison of degrees of crystallinity obtained by different methods.

Compound	Method of sample preparation	% Crystallinity determined by					Reference
		X-ray		IR	Solution calorimetry	Stability at 50°C and 31% RH	
		Herman's method	Using internal standard				
Cellulose	Grinding 0 min	63		59			Nakai et al., 1977
	40 min	49		57			
	8 h	14		38			
	32 h	0		10			
Cephalexin[a]	Grinding 15 min	60	28	12			Otsuka and Kaneniwa, 1983
	30 min	42	28	15			
	1 h	29	17	15			
	2 h	5	4	17			
Cephalothin	Commercial lots	72			93	100	Pikal et al., 1978
	Freeze dried	62			88	100	
	Freeze dried (different batch)	47			54	85	
	Spray dried	37			47	44	

[a]Interpolated from published figures.

crystallinity can be altered by simply changing the grinding time. A decrease in crystallinity with increasing grinding time has been observed with microcrystalline cellulose (Nakai *et al.*, 1977; Huttenrauch, 1978), lactose (Huttenrauch and Keiner, 1976b; Nakai *et al.*, 1982) and cephalexin (Otsuka and Kaneniwa, 1983). In the dry grinding of crystalline materials, considerable energy is supplied of which only a fraction is used in plastic deformation and in the formation of new surfaces (Hersey and Krycer, 1979). The remainder is converted directly into heat or is stored by the material causing progressive lattice disorder resulting in decreased crystallinity. Prolonged grinding can lead to the formation of an amorphous phase (Lin and Somasundaran, 1972) or the creation of an amorphous surface layer (Khodakov and Rebinder, 1961).

Other techniques for decreasing the crystallinity of solids are: (a) preparation of solid dispersions where the crystalline solid is dispersed in a solid state inert carrier or matrix (Chiou and Riegelman, 1971) and (b) the addition of non-toxic impurities during crystallization (Chow *et al.*, 1984). This study is restricted to systems consisting of calcium gluceptate and water.

E. EFFECT OF CHANGES IN DEGREE OF CRYSTALLINITY ON VARIOUS PROPERTIES

The degree of crystallinity of a solid can have a profound influence on its properties. Table III lists some of the changes in the properties of solids induced by changes in the degree of crystallinity. Decreasing crystallinity confers both desirable (eg., increased apparent solubility, dissolution rate, improved mixing etc.) and undesirable (eg., decreased stability, increased adsorption etc.) properties to pharmaceutical solids. As is evident from Table III, the bulk of the work in this area is by Huttenrauch and his co-workers. Two solids studied by these workers are microcrystalline cellulose and lactose. Huttenrauch and Keiner (1979a) dehydrated α-lactose monohydrate by heating it at 125°C under vacuum. Increasing the drying time was said to decrease the crystallinity of anhydrous α-lactose. This work, however raises some questions regarding methodology and interpretation:

1. The suspension density method (discussed in section C.3 in Introduction) was used for evaluating the changes in crystallinity. With increasing drying time, two processes occur simultaneously: (a) progressive dehydration and (b) progressive decrease in crystallinity of the anhydrate. The first process would cause an increase in density of the particles because Huttenrauch and Keiner (1979a) gave the x-ray density of α-lactose monohydrate as

Table III Effect of changes in crystallinity on various properties.

Property	Compound(s)	Effect	Reference
Adsorption of water	Indomethacin	Inversely related to percent crystallinity (linear)	Imaizumi et al., 1980
Adsorption of water	Cephalothin sodium	Inversely related to percent crystallinity (non-linear above 88% crystallinity)	Pikal et al., 1978
Adsorption of water	Two types of cellulose	Increased adsorption in cellulose with lower crystallinity	Huttenrauch et al., 1976
Adsorption of dye	Cellulose	Inversely related to percent crystallinity (linear)	Huttenrauch and Keiner, 1975
Solubility and dissolution rate	Digoxin[a]	Increased solubility and dissolution rate with decreased crystallinity	Florence et al., 1974
Dissolution rate	Potassium perchlorate[a]	Increased dissolution rate with increased dislocation density	Burt and Mitchell, 1981
Solid-state stability	Sodium prasterone sulfate	Inversely related to percent crystallinity	Nakagawa et al., 1982
Wettability	(i) Sulfathiazole[a] (ii) Lactose	Increased wettability with increased disorder in solids	Huttenrauch and Moeller, 1983
Rate and extent of water removal	(i) Citric acid monohydrate[a] (ii) Lactose monohydrate	Increased lattice disorder caused increased rate and extent of water removal	Huttenrauch and Fricke, 1981
Melting	Digoxin[a]	The melting behavior affected by changes in crystallinity	Florence et al., 1974; Chiou and Kyle, 1979

Table III (continued)

Property	Compound(s)	Effect	Reference
Mixing of solids	Norethisterone acetate in lactose	Increased homogenity of powder with increased disorder in lactose (linear)	Huttenrauch and Keiner, 1979b; Huttenrauch et al.,1979
Mixing,incorporation and efficiency of lubricants in tablets	Lactose with magnesium stearate as lubricant	Increased disorder of lactose increased the mechanical stability of tablets	Huttenrauch, 1977b
Properties of suppositories	Polyethylene glycol 4000[a]	Increased density, impact strength and dissolution time with increased crystallinity	Huttenrauch and Fricke, 1979b
Drug release from ointment base	Salycylic acid release from ointment base consisting of polyethylene glycol and mineral oil[a]	Increased release rate of drug with increased degree of order of base	Huttenrauch and Fricke, 1979a
Consistency of ointment base	Two ointment bases: (i)artificial petrolatum[a] (ii)polyethylene gels[a]	Improvement in consistency with increased crystallinity	Huttenrauch et al., 1973

[a]Speculation

1.520 g cm^{-3} compared with 1.547 g cm^{-3} for *a*-lactose. The second process would most likely decrease the density of the particles. It is not clear how the opposing influences of these two processes were distinguished in the suspension cell.

2. Itoh *et al*. (1977) report that heating *a*-lactose monohydrate at 111°C under vacuum resulted in the formation of unstable anhydrous lactose that was highly hygroscopic. Since Huttenrauch and Keiner (1979a) used similar conditions for dehydrating *a*-lactose monohydrate, the formation of an unstable phase is a possibility which was not discussed.
3. In addition to the unstable anhydrate, Lerk *et al*. (1984) report the preparation of two more crystal forms of anhydrous *a*-lactose from *a*-lactose monohydrate. Though Huttenrauch and Keiner report an x-ray density value of 1.547 g cm^{-3} for anhydrous *a*-lactose, no details of the characterization of this material were given.

F. NON-CRYSTALLINE SOLIDS

1. Preparation

Non-crystalline solids of pharmaceutical interest have been prepared by a variety of techniques (Table IV). In each case, the final solid phase was concluded to be non-crystalline from its diffuse x-ray diffraction pattern.

Table IV Preparation of non-crystalline compounds by different methods.

Reference	Compound	Method of preparation of the non-crystalline compound
Otsuka and Kaneniwa, 1983	Cephalexin	By grinding the corresponding crystalline forms
Lerk et al., 1984	α-Lactose monohydrate	
Nakai et al., 1977	Microcrystalline cellulose	
Haleblian et al., 1971	Fluprednisolone	Freeze drying
Pikal et al., 1977, 1978	Several β-lactam antibiotics	
Yarwood et al., 1983	Sodium ethacrynate	
Corrigan et al.,1984	Some thiazide diuretics	Spray drying
Sato et al.,1981	9,3"-Diacetylmidecamycin	
Stagner and Guillory, 1979	Iopanoic acid	Precipitation from solution
Mullins and Macek, 1960	Novobiocin	
Borka, 1974	Indomethacin	Formation of glasses by cooling from the melt
Summers, 1978	Several barbiturates	

2. Pharmaceutical implications

The effect of changes in crystallinity on the properties of some substances was listed in Table III. The following discussion is therefore restricted to a comparison of the properties of some solids in just two discrete states - crystalline and amorphous. In a solid, the energy of the amorphous form is higher than that of the crystalline form. Therefore, the amorphous solid will be more reactive than its crystalline counterpart and will likely have a higher apparent solubility and dissolution rate. The use of the non-crystalline form may therefore be preferred. Mullins and Macek (1960) observed that, for the same dose, x-ray amorphous novobiocin was readily absorbed in dogs and produced therapeutically adequate concentrations while crystalline novobiocin was not absorbed at all. This difference in bioavailability was correlated to the marked differences in the dissolution rate and apparent solubility of the crystalline and amorphous forms in O.1N hydrochloric acid. Amorphous novobiocin in suspension had a tendency to readily convert to the stable crystalline form. This problem may be encountered whenever the metastable form of a compound is used and in such cases steps have to be taken to stop or at least decelerate such a transition. Almirante *et al*. (1960) administered both crystalline and amorphous forms of chloramphenicol stearate to rats and while the amorphous form was hydrolyzed to liberate the antibiotic, the crystalline form was therapeutically inert. The duration of action of

insulin can be controlled by its crystallinity. When reacted with zinc chloride, insulin precipitates as an insoluble complex and depending on the pH, it precipitates either as an amorphous or as a crystalline phase (Hallas-Moller *et al.*, 1952). Prompt Insulin Zinc Suspension (USP XX, 1980d) is described as a suspension of amorphous insulin zinc complex and its effect begins about 1 h after subcutaneous injection, reaches a maximum in 6 h and lasts about 12 to 16 h (Martindale, 1982a). Extended Insulin Zinc Suspension (USP XX, 1980d) consists of a predominantly crystalline solid phase and its effect begins 4 to 6 h after subcutaneous injection, reaches a maximum in about 10 to 19 h and lasts for 30 to 36 h. Intermediate response time and duration of action is achieved through Insulin Zinc Suspension (USP XX, 1980e) which consists of a mixture of crystalline and amorphous insulin in a ratio of approximately 7 parts of crystals to 3 parts of amorphous materials. The effect begins within about 2 h, reaches a maximum in about 8 to 12 h, and lasts for about 30 h.

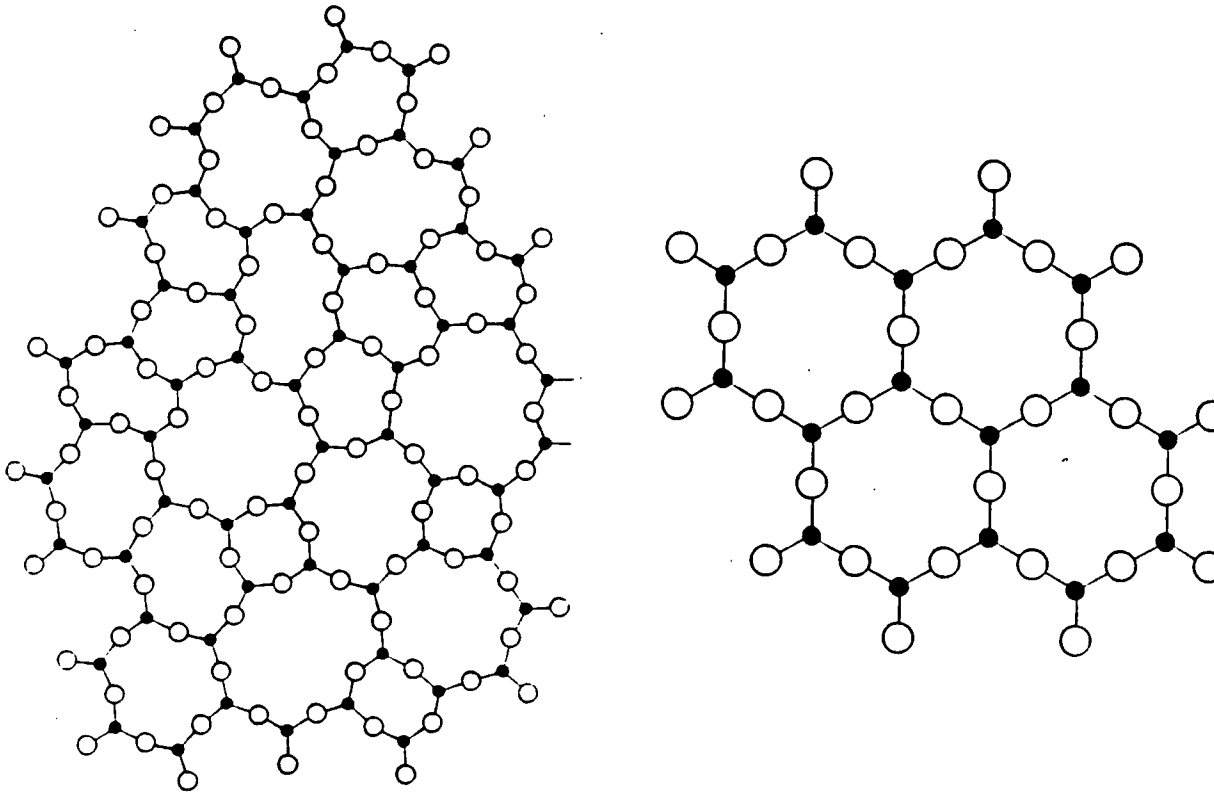
The increased reactivity of non-crystalline solids can lead to problems particularly of chemical stability. In the case of cephalosporins (Pfeiffer *et al.*, 1976; Pikal *et al.*, 1978; Oberholtzer and Brenner, 1979) and potassium penicillin (Mathews *et al.*, 1966 and Pfeiffer *et al.*, 1976) the amorphous forms were qualitatively compared to their crystalline counterparts, and found to be less stable. Pikal *et al.* (1977) concluded that the stability of the amorphous forms of some

cephalosporins (cephalothin sodium, cefamandole sodium and cefamandole nafate) were at least one order of magnitude less than the corresponding crystalline forms. Some drugs are detectably hygroscopic only in their amorphous form. This has been observed in epicillin (Hou and Restivo, 1975) and potassium penicillin (Mathews *et al.*, 1966).

3. Structure of non-crystalline solids

There are broadly two schools of thought about the structure of non-crystalline solids - the "random network" theory of Zachariasen (1932) and the "microcrystallite" theory due to Lebedev (1921). Zachariasen specifically discusses the atomic arrangement in glasses but the arguments are also relevant to other non-crystalline solids like amorphous solids. The periodicity of arrangement of atoms is the characteristic feature of a crystal. Periodicity implies that a structural unit called a unit cell exists which builds up a crystal by being repeated in three directions. Glasses are characterized by an absence of such long range periodicity. However, it is valid to assume that the atoms in glass are linked together by forces essentially the same as in crystals, because over long ranges of temperature, the mechanical properties of a glass and its crystalline counterpart are comparable. The isotropic character of glass is a natural consequence of the absence of symmetry in the network (lattice) because the atomic arrangement will be statistically the same in all directions. The network in glass can be

characterized by an infinitely large unit cell containing an infinite number of atoms. Because of the lack of periodicity, no two atoms are structurally equivalent while in a crystal lattice like that of sodium chloride, all the sodium ions are structurally equivalent i.e. all of the sodium ions have exactly identical surroundings (surface conditions are disregarded). Similarly, all the chloride ions are also structurally equivalent. Since the atoms in glasses are structurally unequivalent, the energy required to detach an atom from the network will be different for each individual atom. With increasing temperature, an increased number of atoms are detached so that the breakdown of the network is a continuous rather than an abrupt phenomenon (i.e. the absence of a sharp melting point is characteristic of amorphous solids). On the other hand, a crystal lattice consists of structurally equivalent atoms, and when the thermal energy reaches a definite value, all the equivalent atoms are simultaneously detached and the crystal network breaks down abruptly. Figure 3a shows, in two dimensions, the lattice of a crystal of composition A_2X_3 while Fig. 3b represents the glass network of the same compound. Warren (1937) worked with vitreous silica (fused quartz) and, from the x-ray results, pictured glassy silica as a random network in which each silicon atom was surrounded tetrahedrally by four oxygen atoms, and each oxygen bonded to two silicon atoms, the two bonds to an oxygen being roughly diametrically opposite. The orientation of one tetrahedral group with respect to a



(a) (b)

Fig. 3 Schematic representation in two dimensions of lattice of a compound of composition A_2X_3 (● atoms of A, O atoms of X): (a) glass and (b) crystal (Zachariasen, 1932).

neighboring group could be practically at random. However, there was a definite scheme of structure involved in that each atom had a definite number of nearest neighbors at a definite distance, but no unit of structure repeated itself identically at regular intervals in three dimensions. Hence the material was not crystalline.

According to Lebedev (1921,1940) glasses are an aggregation of highly dispersed crystallites of 0.7 to 1.5 nm (7 to 15 Å) size range and in spite of their small size are not greatly distorted and preserve to a considerable degree their individual properties. For example, Wagner *et al*. (1968) prepared vapor quenched films of AgCu alloy and found them to be microcrystalline with face-centred cubic structure and a particle size of less than 1.6 nm. Crystals of this size range will have very broad x-ray diffraction lines and may be considered to be x-ray amorphous (Cullity, 1978c).

Warren (1937) studied the x-ray behavior of vitreous silica and found evidence refuting Lebedev's 'microcrystallite' theory. From the x-ray line breadth of fused quartz, he calculated the average particle size to be 0.77 nm. Since the edge of the unit cell of cristobalite (the crystalline form of quartz) is ≃0.7 nm, if Lebedev's theory were valid, then each crystallite should comprise of scarcely more than one unit cell and calling it "crystalline" is of questionable validity. Moreover, if the material did consist of discrete crystallites, then small angle scattering (at 2θ

less than 10°) can be expected. For example, silica gel strongly scatters x-rays up to 10° 2θ due to the existence of discrete particles of 1-10 nm size range. Warren observed no small angle scattering with fused quartz and concluded that the scheme of bonding was essentially continuous and fitted the model of Zachariasen.

Nevertheless, the structure of non-crystalline materials is still only incompletely understood (Zarzycki, 1977). Since diffraction studies are insensitive to fine details of structure, they cannot be used to distinguish between random network and microcrystallite models. High resolution electron microscopy constitutes an improvement over the diffraction methods, but it provides only two-dimensional information and therefore cannot provide an unequivocal distinction between the two models. According to Zarzycki (1977), only by modelling can three-dimensional information be obtained and thus the structures of the disordered state understood. Though the controversy is far from resolved, Gaskell (1977) believes that a referendum on the subject of microcrystallite versus random network as the most appropriate model for the structure of "ideal" glasses would result in an overwhelming vote in favor of the latter.

4."Polymorphism" in non-crystalline solids

Polymorphism is the ability of a compound to crystallize as more than one distinct species (Haleblian and McCrone,

1969). According to Roy (1970), analogous "polymorphs" with different non-crystalline solid structures are possible. For example, he reports the preparation of reaction amorphized, shear amorphized and radiation amorphized silicon dioxide as well as its preparation as a glass, as a desiccated gel and as a vapor deposited non-crystalline solid phase. Though he states that these phases have different properties, the differences were not discussed. Roy (1970) also suggests that non-crystalline solid phases with minor differences in structure seem possible. An example is a glass cooled over a 30 day period and another quenched in 2-3 seconds over the same temperature range. According to Finney (1977) a variety of amorphous structures are possible depending upon the preparation method, presence of impurities and the nature of the molecules concerned. In spite of the multitude of structures, Finney believes that all amorphous substances can be treated within the same conceptual framework of a random network of linked molecules (Zachariasen's model). Pikal *et al*. (1978) observed significantly different heat of solution values between spray dried and freeze dried cefamandole nafate. They believed that the spray dried amorphous material was an annealed form of the amorphous material and suggested the possibility of differences in structure between different amorphous samples of the same compound.

According to Huttenrauch's (1978) concept of crystallinity of solids, which was discussed earlier, the

transition from the crystalline to the non-crystalline state is due to a gradual and continuous increase in disorder of the lattice. Thus there could be samples with extensive but not complete lattice breakdown. For example, spray dried and freeze dried samples might both have a highly but not completely disordered lattice. Small differences in their properties (e.g., in enthalpies of solution) could be due to differences in the extent of lattice disorder and it is unnecessary to invoke the concept of "polymorphism" in the amorphous state.

G. INTRODUCTION TO CALCIUM GLUCEPTATE

Calcium gluceptate is used in the treatment of calcium deficiency (Martindale, 1982b). Complexed with technetium, it is used in nuclear medicine as an organ scanning agent (Chi *et al.*, 1978). It is very soluble in water (Suryanarayanan and Mitchell, 1984) and is particularly useful in the preparation of veterinary products containing a high calcium concentration[1]. When first introduced, calcium gluceptate was an approximately equal mixture of the calcium salts of D-glycero-D-gulo heptonic acid (Fig. 4a) and D-glycero-D-ido heptonic acid (Fig 4b). The mixture of α and β epimers was anhydrous and amorphous to x-rays (Suryanarayanan and Mitchell, 1981). Aqueous solutions (27%w/w) prepared with this material were stored for two years and were stable

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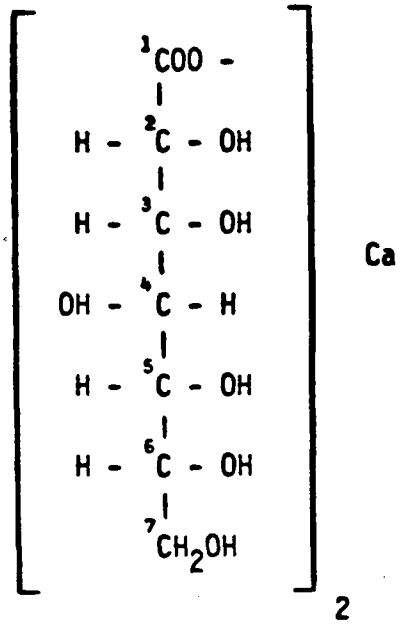
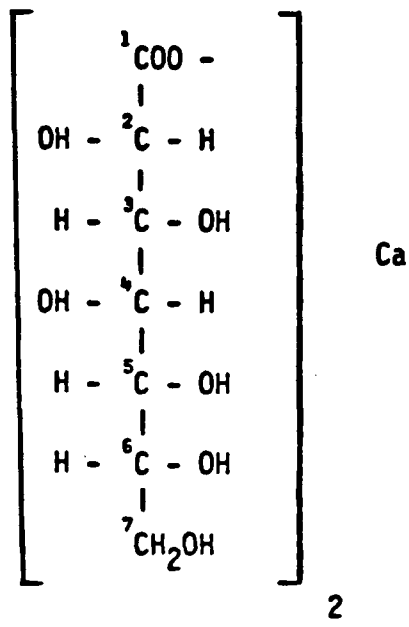
[1]Product information of calcium gluceptate, Pfanstiehl Laboratories, Waukegan, IL, USA.



(a)



(b)

calcium D-glycero-D-gulo-heptonate

or calcium α-D-gluco-heptonate

or calcium gluceptate USP

calcium D-glycero-D-ido-heptonate

or calcium β-D-gluco-heptonate

Fig. 4 Structures of (a) calcium α-glucoheptonate and (b) calcium β-glucoheptonate.

(Pfanstiehl, lot 7311; Table V). Calcium gluceptate became official in the USP in 1976 (USP XIX, 1976) where it was described as calcium *a*-glucoheptonate (Fig. 4a). In order to comply with pharmacopeial specifications, the manufacturers were required to change their synthetic and recrystallization procedures in order to exclude the β-form. This apparently resulted in an increase in the relative proportion of the *a* epimer (e.g., Givaudan, lot R 3679 BA and Italsintex, lot R 1432 TJ, Table V), but the material still consisted of a mixture of *a* and β epimers. Solutions prepared with these materials by Suryanarayanan and Mitchell (1984) were not stable and precipitated within 9 days. The precipitate was found to be crystalline calcium gluceptate hydrate with 3 1/2 molecules of water of crystallization. The 27%w/v aqueous solutions of calcium gluceptate are supersaturated with respect to calcium gluceptate hydrate resulting in the precipitation of the hydrate. The Givaudan material has an apparent water solubility > 200% w/v (its equilibrium solubility could not be determined) while the equilibrium solubility of the precipitated hydrate is ≃ 3% w/v. Muller *et al*. (1979) reported that the problem of precipitation from solution was encountered from late 1976 onwards (Givaudan, Table V). It therefore appears that approximately equal proportions of the *a* and β epimers are stable in solution but that stability decreases with the increase in the relative proportion of the *a* epimer. It seems possible that the β epimer has a stabilizing effect on the solution. When pure

Table V Relationship between the proportions of α and β epimers and the stability of aqueous solutions of calcium gluceptate (27% w/v) stored at room temperature (Suryanarayanan and Mitchell, 1984).

Calcium gluceptate source	Epimers in calcium gluceptate, (%)		Precipitation begins, (days)
	α	β	
Pfanstiehl, lot 7311	52	41	Stable
Givaudan, lot R 3679 BA	72	28	8
Italsintex, lot R 1432 TJ	72	28	2
Givaudan[a]	77	23	4-6 months
Pfanstiehl, lot 12953-D	100	0	<1

[a]Reported by Muller et al., 1979; these are formulations and the initial calcium gluceptate concentration is not given.

calcium *a*-gluceptate became available from Pfanstiehl (lot 12953-D, Table V), aqueous solutions were found to be extremely unstable and precipitated within a day of preparation.

The role of seed crystals in the precipitation of calcium gluceptate solutions has also been studied. Electron microscopic studies by Muller *et al*. (1979) revealed that solutions prepared from late 1976 onwards contained seed crystals of about 15μm size which induced crystallization. Solutions prepared prior to that time did not contain the seed crystals and were stable. Suryanarayanan and Mitchell (1984) filtered (0.22μm membrane filter) calcium gluceptate solutions and found that the time for precipitation increased, suggesting that filtration excluded some but not all of the seed crystals. When the solutions were filtered through a 0.1 μm filter and then examined in a laser light scattering photon correlation spectrometer[2], there were no particles of a measurable size (i.e. >5 nm in radius) suggesting that all the seed crystals had been excluded. Autoclaving at 121°C for 20 min resulted in stable solutions in all cases, suggesting that autoclaving destroyed the seed crystals. It therefore seemed that both seed crystals and the relative proportions of the *α* and *β* epimers played a role in the stability of calcium gluceptate solutions.

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[2]Unpublished work. The experiments were conducted by Dr. T. Whateley, Department of Pharmacy, University of Strathclyde, Glasgow, UK.

More recently, calcium gluceptate has been commercially available as a crystalline hydrate (3 1/2 molecules of water of crystallization). Holstein (1980) who patented the manufacturing procedure of calcium gluceptate (Holstein, 1962), reports that preparation of calcium gluceptate in the amorphous form has become impossible due to the presence of seed crystals or some other factor initiating crystallization. Holden and Singer (1960) described a similar incident in which ethylenediamine tartrate crystals having been grown in a factory for a year without any problems were suddenly contaminated by the simultaneous growth of ethylenediamine tartrate monohydrate crystals. Crystallization of the monohydrate was attributed to seed crystals of the monohydrate though it was not known how the seed crystals had suddenly formed. In the case of calcium gluceptate, the commercially available material has changed abruptly from an amorphous anhydrate with an apparent aqueous solubility > 200% w/v, to a crystalline hydrate with an equilibrium water solubility of ≃3% w/v. It is evident that the difference in crystallinity and/or the state of hydration is responsible for this dramatic change in properties. If crystalline calcium gluceptate hydrate can be dehydrated and rendered amorphous, its apparent solubility can be expected to increase markedly and approach that of amorphous anhydrous calium gluceptate.

H. OBJECTIVES

The objectives of this investigation were:

1. To characterize the solid-state properties of calcium gluceptate obtained from different sources and at different times from the same source.
2. To study the solid-state and solution phase transitions of calcium gluceptate and to prepare 'stable' pharmaceutical solutions.
3. To evaluate the various concepts of crystallinity of solids using calcium gluceptate as a model compound.

EXPERIMENTAL

A. APPARATUS

Autoclave, AMSCO general purpose, American Sterilizer Company.

Bioquest biological cabinet, Beckton Dickinson.

Borosilicate glass tubes, Kimax, Owens-Illinois with polytetrafluoroethylene-lined screw cap.

Cahn electrobalance, Gram, Ventron Corporation.

Constant temperature bath, Magni Whirl, Blue M Electric Company.

Density meter, DMA 45, Paar.

Differential interference contrast microscope, Model R, Nikon.

Differential scanning calorimeter with effluent gas analyzer, DSC-1B, Perkin Elmer.

Freezer (-76°C), UC 105, Kelvinator.

Freeze drying unit, Virtis Company.

Gas chromatograph with a flame ionization detector, model 5830 A, Hewlett Packard and a GC terminal, model 18850 A, Hewlett Packard.

Hot-stage, FP2, Mettler.

Incubator, Isotemp, Fisher.

Mechanical agate mortar and pestle, Pulverisette 2, Fritsch.

Oven, Shel-lab model 22, Sheldon.

pH meter, model 26, Radiometer.

Polarizing microscope, Standard 14, Zeiss.

Proportional temperature controller, model 76, YSI Company; with vinyl probe, model 402, YSI Company.

Rotating mixer, Dyna-mix, Fisher.

Solution calorimeter, model 1451, Parr.

Sterifil filtration system, Millipore.

Surface area analyzer, Quantasorb Sorption System, Quantachrome.

Syringe filter, o.22 μm Nalgene, Nalge Company.

Thermogravimetric analyzer, model 950, Du Pont, with differential thermal analyzer, model 900, Du Pont.

Vacuum oven, National Appliance.

Vacuum pump, Vac Torr S 35, General Electric.

Vials, Reacti-Vial, Pierce.

Water pump, R21, Haake.

X-ray diffractometer with a xenon proportional counter, wide angle, Philips.

B. MATERIALS

Acetonitrile, HPLC grade, Caledon.

Amberlite IR-120 ion exchange resin, Mallinckrodt.

Ammonium chloride, BDH.

Calcium gluceptate, Pfanstiehl, lot 7311.

Calcium gluceptate, Sigma, lot 126C-0121.

Calcium gluceptate, Givaudan,lot R 3679 BA.

Calcium gluceptate, Italsintex, lot R 1432 T J.

Calcium gluceptate, Pfanstiehl, lot 12953-D.

Calcium gluceptate, Pfanstiehl lot 13313-E.

Calcium gluceptate, Pfanstiehl, lot 14772.

Calcium gluceptate, Pfaltz and Bauer, lot C01300.

Calcium gluceptate, Princess Margaret Hospital (PMH).

Calcium gluceptate, Merck, ST-16944, RM-45940.

Carbon tetrachloride, ACS grade, BDH.

Colloidal silicon dioxide (Cab-O-Sil), Cabot Corporation.

Cupric chloride dihydrate, Matheson Coleman and Bell.

3% Cyanopropylphenylmethyl silicone (OV-225) on Chromosorb W(HP), 100-120 mesh, Western Chromatography.

Ethylene dibromide, BDH.

Formic acid, ACS grade, Fisher.

α-D-Glucoheptonic acid γ-lactone, Aldrich.

Hydrochloric acid, ACS grade, Allied Chemical.

Indium, Goodfellow Metals.

Lithium fluoride, Fisher.

Magnesium chloride hexahydrate, BDH.

Methanol, HPLC grade, Fisher.

Methylene chloride, HPLC grade, Caledon.

Phosphoric acid, ACS grade, Allied Chemical.

Phosphorus pentoxide, ACS grade, BDH.

Potassium acetate, BDH.

Potassium carbonate, BDH.

Silica gel, Davison Chemical.

Sodium dichromate dihydrate, BDH.

Sodium hydroxide, ACS grade, Fisher.

Sodium nitrite, Allied Chemical.

Trimethylsilylimidazole in pyridine (TRI-SIL 'Z'), Pierce.

Tris(hydroxymethyl)aminomethane, Parr.

Water, distilled.

Zinc sulfate heptahydrate, ACS grade, BDH.

C. CHARACTERIZATION OF CALCIUM GLUCEPTATE

Calcium gluceptate (Pfanstiehl, lot-13313 E; crystalline hydrate containing 3 1/2 molecules of water of crystallization), I, was used as received or it was dried at 60°C, under vacuum (pressure < 130 Pa) for 16 h (USP XX, 1980c) to yield the anhydrate, II. For the phase transition studies, in addition to I and II, x-ray amorphous, anhydrous calcium gluceptate, III (Pfanstiehl, lot 12953-D) was also used. The characterization of I and II is described in the following pages. III was characterized earlier (Suryanarayanan and Mitchell, 1984).

The samples of calcium gluceptate obtained from all other sources were used as received to compare some of their solid-state properties

1. Gas chromatography (GC)

The USP monograph describes calcium gluceptate as calcium *α*-glucoheptonate (USP XX, 1980c) but the analytical procedures for the identification and assay of calcium gluceptate are incapable of distinguishing between the calcium salts of *α* and *β*-D-glucoheptonic acids. Suryanarayanan and Mitchell (1984) obtained calcium gluceptate samples from different commercial sources that consisted of varying proportions of calcium *α*-glucoheptonate and calcium *β*-glucoheptonate. A GC technique was developed to separate and determine the relative proportions of these two epimers. Their identity was

established by gas chromatography-mass spectrometry (GC-MS). Calcium gluceptate solution was passed through a column of cation exchange resin in order to convert it to α and β-D-glucoheptonic acids and the eluant was lyophilized and repeatedly treated with concentrated hydrochloric acid. This caused complete conversion of the glucoheptonic acids to their corresponding γ-lactones (1,4-lactones) which were trimethylsilylated with trimethylsilylimidazole in pyridine. There was a baseline separation of the derivatized α-D-glucoheptonic acid γ-lactone and β-D-glucoheptonic γ-lactone which had retention times of 9.2 and 7.7 min respectively. GC-MS showed that the above two compounds had similar mass fragmentation patterns. A commercially available reference sample of α-D-glucoheptonic acid γ-lactone when subjected to GC-MS analysis under the same conditions, had a retention time of 9.2 min and also the same fragmentation pattern as the above two compounds. Hence, the identity of the compound eluting at 9.2 min was confirmed. Since the compound eluting at 7.7 min had a similar mass fragmentation pattern, it was assumed to be the trimethylsilyl derivative of β-D-glucoheptonic acid γ-lactone.

All samples of calcium gluceptate were analyzed using this GC procedure.

2. Powder x-ray diffraction

Samples were exposed to Ni-filtered CuKα radiation (36 kV x 16 mA) at a scanning rate of $1°2\theta$ min^{-1} in a wide angle x-ray diffractometer over a range of 2θ from 10° to 40°.

3. Thermal methods

3.1 Thermogravimetric analysis (TGA)

The weight loss on heating I up to 140°C at 10°C min^{-1} was determined by TGA.

3.2 Differential scanning calorimetry (DSC)

Samples of about 1 to 5 mg were weighed on an electrobalance directly into aluminum sample pans. Scans were made at various rates using standard (open) pans, volatile (closed) pans and volatile pans with a 0.1 to 0.2 mm pinhole. Vaporization of the water of crystallization from the standard pans and the volatile pans with a pinhole was detected using the effluent gas analyzer and was estimated quantitatively by weighing the pan after each endothermic peak. The calorimeter was calibrated with samples of indium.

In all cases, the peak temperature i.e. the point on the temperature scale of maximum deviation from the baseline was reported. The temperatures at which phase transitions occurred depended on the scanning rate and all reported transition temperatures are for a scanning rate of 10°C min^{-1}.

3.3 Thermomicroscopy

Samples were mounted dry or in mineral oil on a glass slide and heated on a hot stage while being observed under a microscope. The temperatures at which transitions occurred were recorded.

4. Equilibrium solubility

Excess of I was added to 20 mL of water contained in a 100 mL volumetric flask. A few such flasks were mechanically rotated in water baths set at 25.5, 31.5, 34.0 and 37°C. Aliquots were drawn periodically into a warm syringe, membrane filtered, weighed and diluted with a known weight of water. The density of the diluted solutions was determined in a density meter thermostatically controlled at 25°C.

Details of the calibration of the density meter are given in section F.3 (p.65) of Experimental. A standard curve was plotted by preparing solutions of I in the concentration range of 0.009 to 0.055 molal and determining their densities, Fig.5. The concentration of I in the diluted aliquots was determined from the standard curve. Equilibrium was assumed when successive aliquots showed no difference in the concentration of I.

Because of the observed linear relationship between concentration and density, the intercept on the y-axis (Fig. 5) should be equal to the density of water at 25°C. The

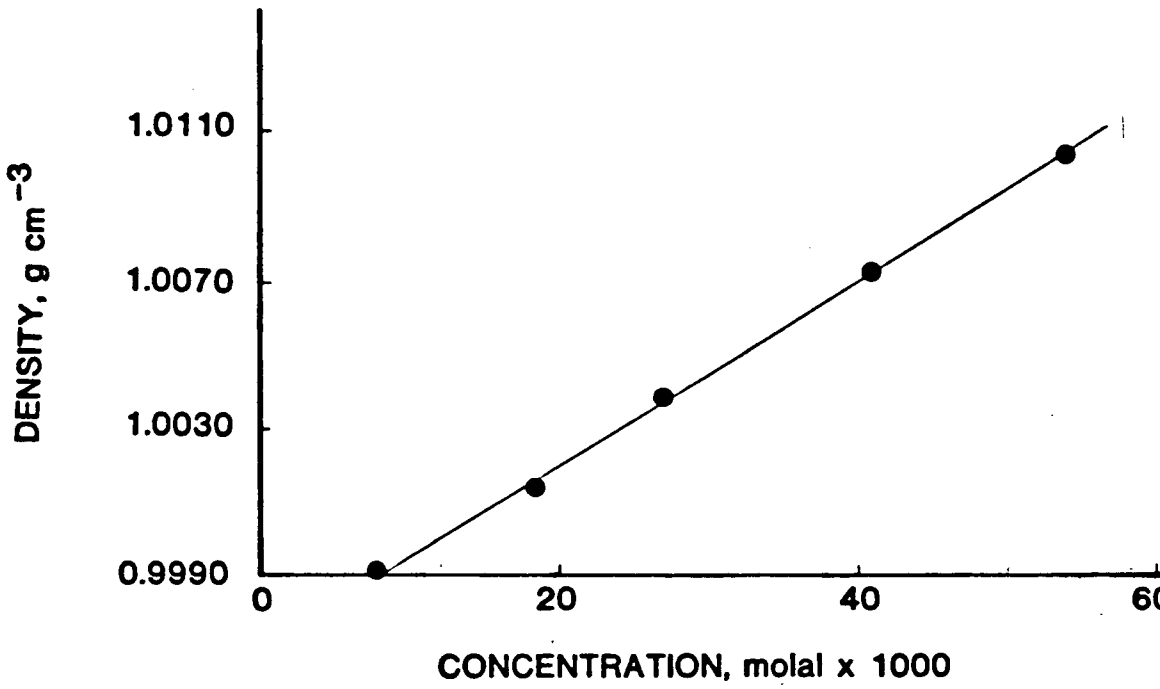

Fig. 5 Standard curve relating the concentration of aqueous solutions of I with their densities.

experimental intercept of 0.9970 g cm^{-3} was close to the reported density of 0.9971 g cm^{-3} for water at 25°C (CRC Handbook, 1983).

D. TREATMENT OF CALCIUM GLUCEPTATE

I and II were subjected to the following treatments to study possible phase transitions.

1. Grinding

Both I and II were ground in a mechanical agate mortar and pestle for varying times. I was ground for up to 1 h and II for up to 4 h. The temperature rise in the sample during grinding was not measured, but for long grinding times, the machine was stopped intermittently to limit any temperature rise.

1.1 Effect of grinding on apparent solubility

Attempts to determine the maximum solubilities of unground and ground II were unsuccessful (section A.5 of Results and Discussion, p.82). Hence, the apparent solubilities of unground and ground I and II were determined. Each sample was added to about 0.5 mL of distilled water in a culture tube and dissolved by vigorous shaking at room temperature (≃22°C) using a vortex mixer. At the first sign of persistent turbidity, the addition of solid was stopped and the solution was weighed. The water was evaporated off at 60°C

under vacuum until the residual solid reached a constant weight.

It is recognized that the apparent solubility is a kinetic property and that the values of apparent solubility obtained would depend on the experimental method. While determining the apparent solubility of II, the first sign of persistent turbidity could be due to: (i) the solution becoming saturated with II and excess II remaining undissolved and/or (ii) the solutions becoming supersaturated with respect to I resulting in its precipitation. The second possibility seems more likely because the appearence of turbidity was followed immediately by copious precipitation of I.

1.2 Effect of grinding on surface area

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

1.3 Effect of grinding on powder x-ray diffraction pattern

The ground samples were subjected to powder x-ray diffraction studies under conditions described in section C.2 (p.52) of Experimental.

2. Freeze drying

About 1 g of I was dissolved in 50 mL of water, the solution was frozen at -76°C and dried in a freeze dryer. The freeze dried material was stored in a glass desiccator containing phosphorus pentoxide until used.

3. Constant humidity studies

Chambers of constant relative humidity (RH) ranging from 9 to 90% were obtained by preparing saturated aqueous solutions of phosphoric acid (9% RH), potassium acetate (20% RH), magnesium chloride hexahydrate (33% RH), potassium carbonate (43% RH), sodium dichromate dihydrate (52% RH), sodium nitrate (66% RH), cupric chloride dihydrate (68% RH), ammonium chloride (79% RH) and zinc sulfate heptahydrate (90% RH) in glass chambers (desiccators). All the glass chambers were stored in an incubator at 25°C. Phosphorus pentoxide was used to obtain a chamber of 0% RH. Accurately weighed amounts of I, II and III were placed in each chamber. The weight changes were monitored periodically until the samples attained constant weight. The percent weight change was then calculated and the solid phase identified by powder x-ray diffraction.

E. PREPARATION OF STABLE CALCIUM GLUCEPTATE SOLUTIONS

Table V shows that a 27% w/v solution of calcium gluceptate in water prepared with one particular sample of calcium gluceptate (Pfanstiehl, lot 7311) was stable during

two years of storage. Our objective was to use I to prepare stable aqueous solutions of calium gluceptate of similar concentrations. This was not possible because of the low aqueous solubility of I at room temperature (see section A.5 in Results and Discussion, p.80). However, II could be successfully used to prepare these solutions (section A.5 in Results and Discussion) but the solutions were highly unstable and precipitated on storage. Suryanarayanan and Mitchell (1984) reported that membrane filtration increased the time before precipitation occurred while autoclaving resulted in stable calcium gluceptate solutions. Several solutions containing between 20 and 27% w/v II in water were prepared. Some were filtered through a filter paper (Whatman Number 1) while others were membrane filtered (0.22 μm). The control batch was left unfiltered. All the solutions were autoclaved at 121°C for 20 min.

F. DETERMINATION OF DEGREE OF CRYSTALLINITY OF ANHYDROUS CALCIUM GLUCEPTATE

Degree of crystallinity determinations were carried out only on ground samples of II. For a quantitative determination of percent crystallinty, it is necessary that 100% crystalline and 0% crystalline (amorphous) reference standards be selected. Unground II was chosen as the crystalline standard (100% crystallinity) and II ground for 4 h was used as the amorphous standard (0% crystallinity). Their powder x-ray diffraction patterns are shown in Fig. 6a and Fig. 6b

respectively.

1. Powder x-ray diffraction

The powder x-ray diffraction conditions are described in section C.2 (p.52) of Experimental. 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. 6a). Various proportions of the crystalline and amorphous standards were mixed to yield reference samples of known crystallinity from 0% to 100%. To an accurately weighed amount 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). 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 the reference samples of known crystallinity. This ratio was plotted as a function of percent crystallinity and used as a standard curve (Fig. 7). Fig. 8 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

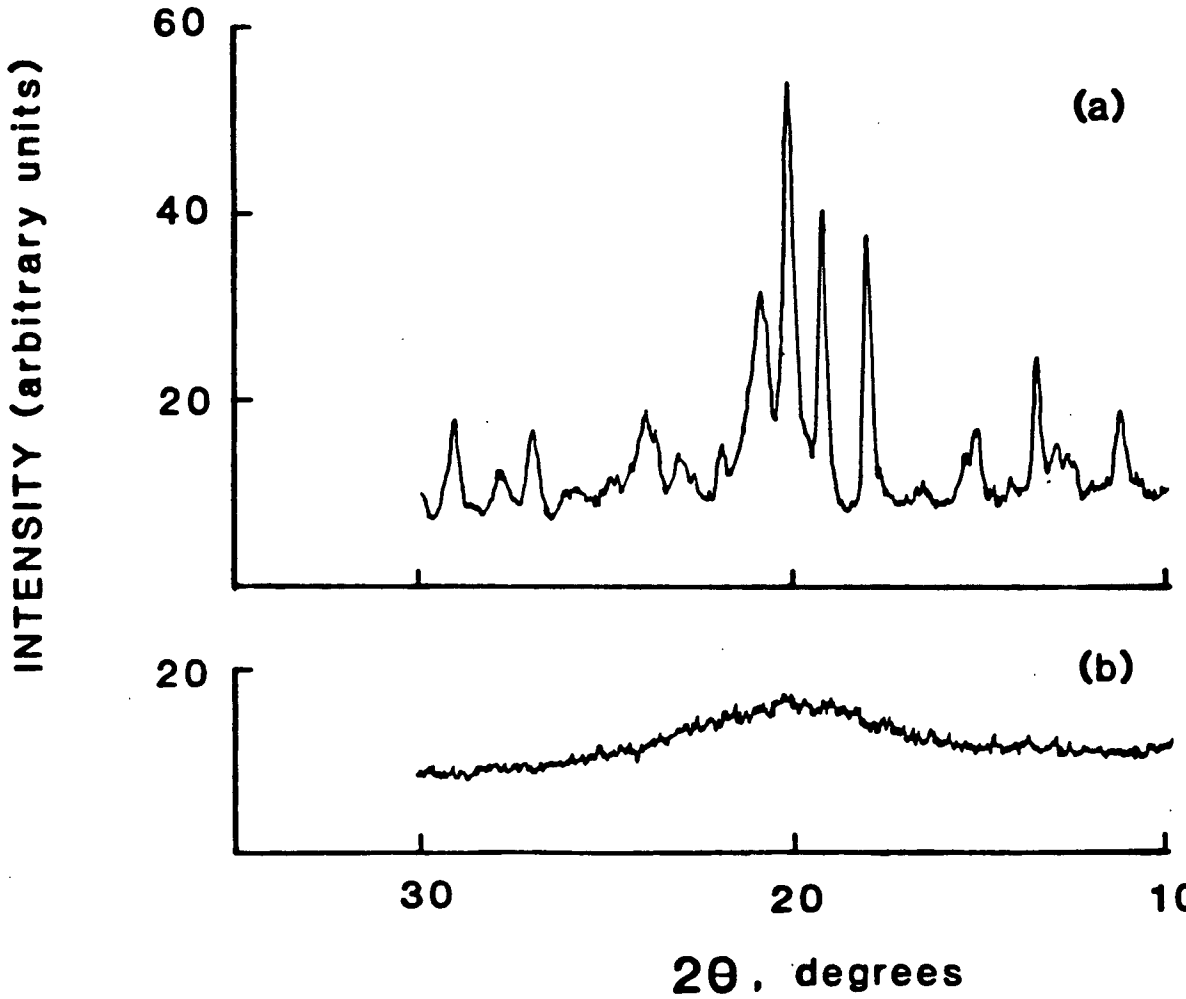

Fig. 6 Powder x-ray diffraction patterns of (a) II (100% crystalline standard) and (b) II ground for 4 h (0% crystalline standard).

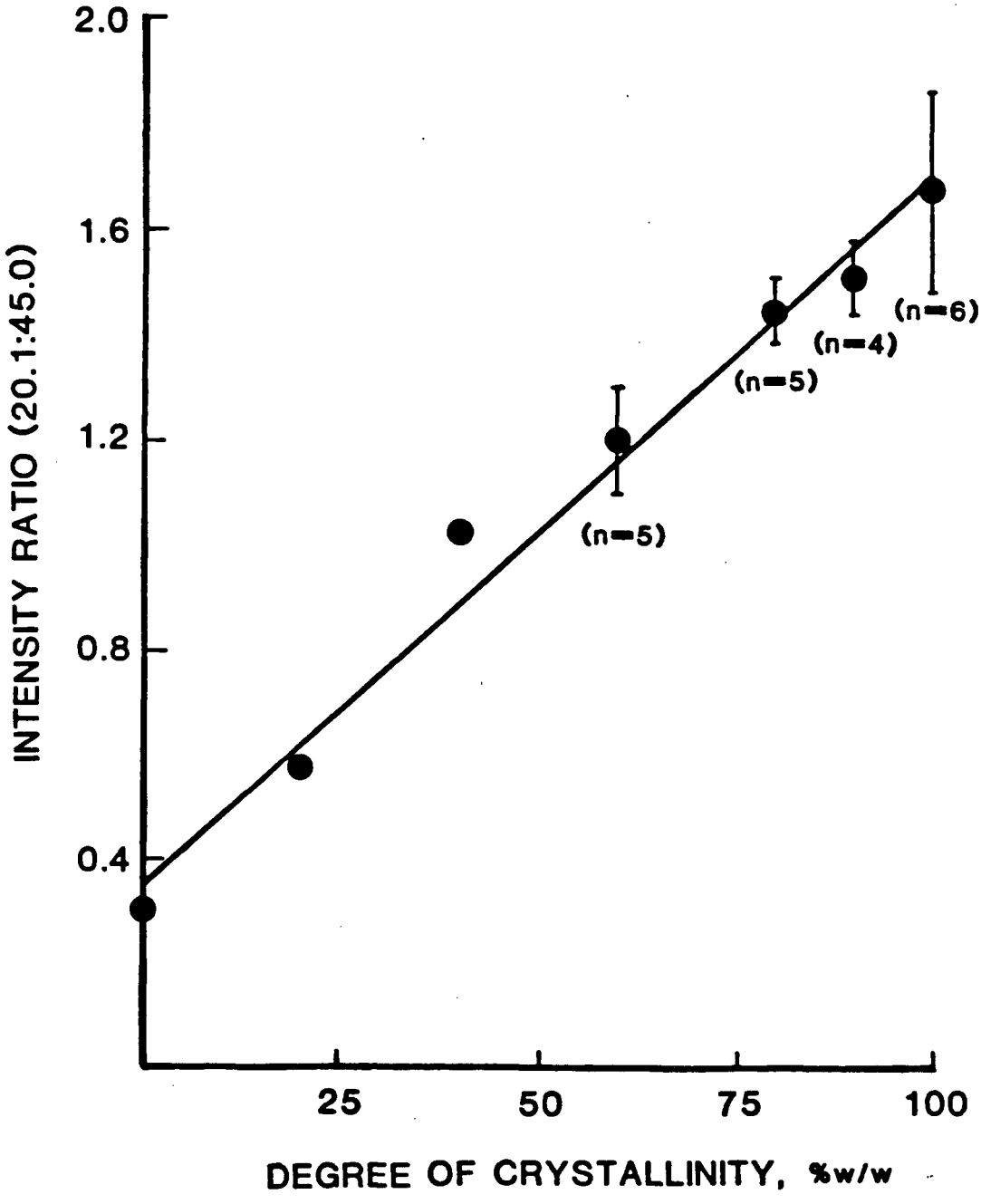

Fig. 7 Standard curve relating the degree of crystallinity of II and intensity ratio of the x-ray diffraction peak of II at 20.1° 2θ to that of lithium fluoride at 45° 2θ. Mean ± standard deviation are shown for selected values; other values are averages of two determinations.

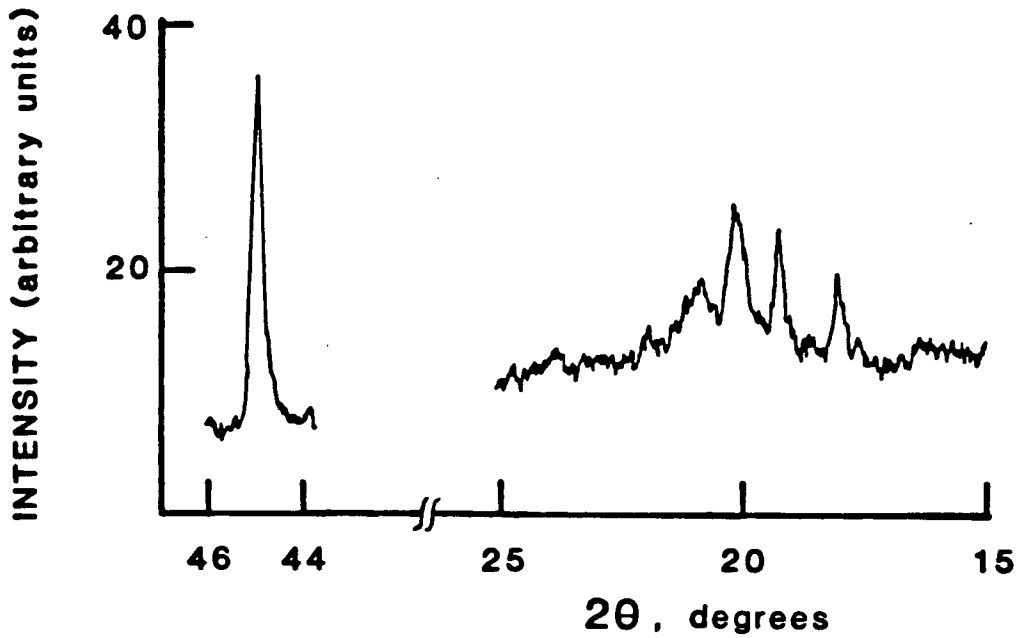

Fig. 8 Powder x-ray diffraction pattern of 20% crystalline II containing 11% w/w lithium fluoride as the internal standard.

of crystallinity at each grinding time was determined from the standard curve.

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 100% 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. 7).

Lithium fluoride was chosen as an internal standard because: (a) its x-ray diffraction peaks did not interfere with those of II, (b) it belongs to the highly symmetrical cubic crystal system and therefore has only few but intense diffraction peaks and (c) its linear absorption coefficient is 31.3 cm^{-1} compared to 35.3 cm^{-1} for II (calculations in Appendix I). From microscopic examination, particles of

lithium fluoride were found to be of less than 5 μm size. Particles of II were also extremely small in size (calculated hypothetical particle size given in Table XI, p.106). The similar linear absorption coefficient values of II and lithium fluoride and the fact that the particle size of both phases was very small suggested that the intensity of diffracted radiation would be negligibly affected by microabsorption (Cullity, 1978d). The density of lithium fluoride is 2.640 g cm^{-3} (Merck Index, 1983a) while that of II is 1.662 g cm^{-3} (Table XI, p.106). Ideally, the compound under investigation and the internal standard should have similar densities so that mixtures remain homogenous after mixing.

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 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.

2. Solution calorimetry

Heats of solution were determined at room temperature (≃22°C) using a solution calorimeter, 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. 9). 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.

3. 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.

Samples of II ground for different times were redried at 60°C under vacuum to constant weight. Each sample was

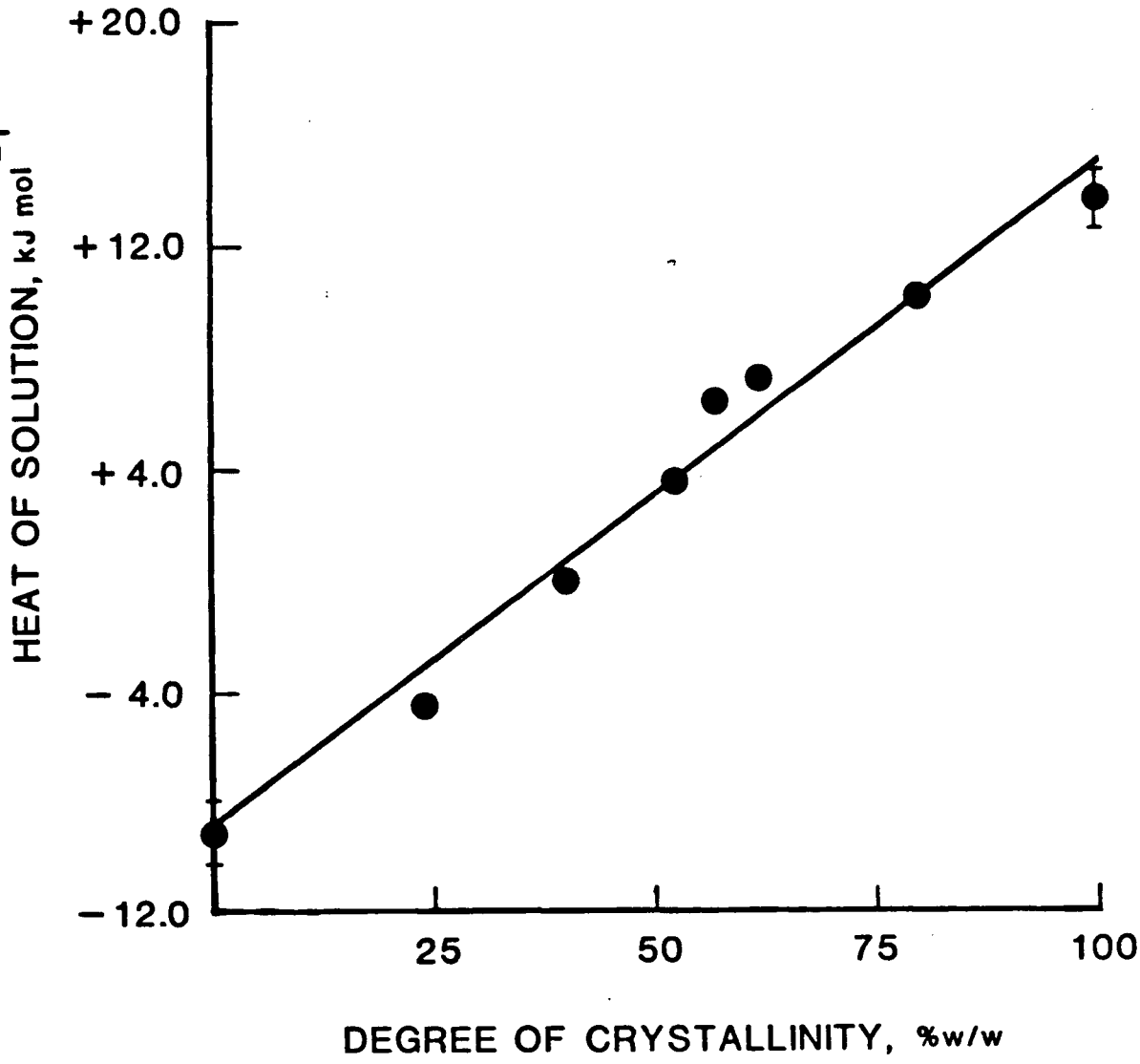

Fig.9 Standard curve relating the degree of crystallinity and the heat of solution of II in water at room temperature (≈22°C). The mean ± standard deviation of 100% and 0% crystalline standards (n=4) are shown.

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 then 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. The tube was closed tightly with a polytetrafluoroethylene-lined screwcap and transferred to a jacketed cell containing water (Fig. 10). Water was pumped from a thermostatically controlled water bath through the double-wall of the cell and then through the outer jacket surrounding the oscillating tube of a digital density meter. 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 g cm^{-3}), 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 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 cm^{-3}) were: acetonitrile 0.7823 [0.7822 (Lange's

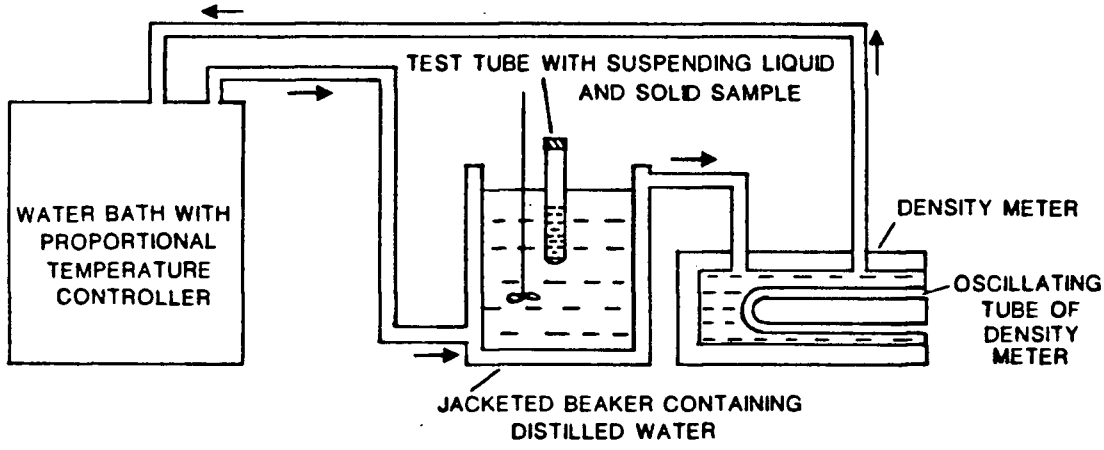

Fig. 10 Schematic diagram of apparatus for suspension density determinations. Arrows indicate the direction of water flow.

Handbook of Chemistry, 1979a)], methanol 0.7920 [0.7915 (Merck Index, 1983b)] and methylene chloride 1.3255 [1.3255 (Merck Index, 1983c)], 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. 10, 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. 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 using Eq. (6) discussed in section C.3 (p.15) of Introduction. The reported percent crystallinity values of ground samples (Table XI) are averages of three determinations.

4. Differential scanning calorimetry(DSC)

Unlike the above three techniques which were used to quantitate the crystallinity of ground II, DSC was used qualitatively to determine the grinding time that causes the complete disappearance of the melting endotherm. For these

studies, standard pans were used and the heating rate was $10^{\circ}C\ min^{-1}$.

5.Polarized light microscopy

Samples of amorphous anhydrous calcium gluceptate (III), unground II and II ground for varying times were mounted in mineral oil and examined by means of a polarizing microscope for birefringence.

RESULTS AND DISCUSSION

A. CHARACTERIZATION OF CALCIUM GLUCEPTATE

1. Gas chromatographic analysis

GC analysis of I by the method of Suryanarayanan and Mitchell(1984) gave a single compound with a retention time of 9.2 min. It therefore consisted of calcium α-glucoheptonate only and thus complied with the USP XX specifications. The same retention time was obtained for II showing that drying at 60°C under vacuum for 16 h did not cause any detectable sample degradation.

2. Powder x-ray diffraction

The powder x-ray diffraction patterns of I and II are shown in Fig. 15a (p.90) and 6a (p.60) respectively. II was obtained from I by dehydration (see section C in Experimental). According to Carstensen (1973a), there are three possible solid-states after dehydration: (i) the crystal lattice is identical to that of the original hydrate, (ii) the residue has a different crystal lattice and (iii) the dehydrated material is amorphous. Since I and II diffract x-rays at nearly the same 2θ values (Table VI), it is apparent that the crystal lattice of crystalline calcium gluceptate remains essentially unchanged on dehydration.

Table VI Powder x-ray diffraction data listing the eight most intense lines of I, the intensities of the corresponding lines of II and the eight most intense lines of the precipitate obtained from a solution of II.

I		II		Precipitate from a solution of II	
Interplanar spacing, d(Å)	Relative intensity, I/I_0(%)	Interplanar spacing, d(Å)	Relative intensity,[a] I/I_0(%)	Interplanar spacing, d(Å)	Relative intensity, I/I_0(%)
2.43	31	2.43	28	2.43	28
2.98	27	2.96	23	2.97	22
3.35	40	3.34	34	3.35	34
3.85	44	3.83	56	3.83	46
3.90	31	3.88	23	3.90	18
4.13	38	4.11	47	4.13	34
4.33	100	4.31	100	4.31	100
5.34	27	5.37	36	5.33	33

[a] The eight most intense lines of II do not correspond with the eight most intense peaks of I but, except for minor differences, the powder x-ray diffraction patterns of I and II are very similar (see Suryanarayanan and Mitchell, 1984).

3. Thermogravimetric analysis

The weight loss on heating I up to 140°C was 11.3% w/w. According to the USP (USP XX, 1980c), calcium gluceptate can exist as an anhydrate or as a hydrate containing 2 molecules or 3 1/2 molecules of water of crystallization. The observed weight loss was close to the theoretical value of 11.4% w/w for the complete dehydration of calcium gluceptate hydrate containing 3 1/2 molecules of water of crystallization.

Attempts to determine the water content of I and II by the Karl Fischer method were unsuccessful. A number of different solvents(e.g., dimethyl sulfoxide, pyridine) were used to dissolve the solids but a satisfactory titration end point was not obtained with any of them.

4. Differential scanning calorimetry and thermomicroscopy

When I was heated in standard pans, there were two endotherms at ≃ 121°C and ≃144°C with a weight loss after the first endotherm of ≃11.2% w/w which was close to the weight loss obtained by TGA (Fig. 11a). Since this endotherm was accompanied by a peak in the effluent gas analyzer, it must be due to simultaneous dehydration and vaporization of water (Scheme Ia). The second endotherm caused no change in effluent gas concentration and was most likely due to melting of the anhydrate. Thermomicroscopy of I mounted dry or in mineral

oil, confirmed that melting occurred at ≃145°C. Based on GC, x-ray and thermal analyses (TGA, DSC and thermomicroscopy), I was identified as crystalline calcium gluceptate hydrate containing 3 1/2 molecules of water of crystallization.

Heating I in volatile pans with or without a pinhole resulted in thermal curves that were markedly different from those obtained by heating in standard pans. Because of the unrestricted nature of standard pans, a hydrate can lose water at any temperature where the vapor pressure of water surrounding the solid is less than the equilibrium vapor pressure of the hydrate at that temperature. The drifting baseline of the dehydration peak (Fig. 11a) suggests a gradual water loss when I is heated in standard pans.

When I was heated in a volatile pan, there was only one endotherm at ≃117°C . This is close to the temperature of dehydration of I in standard pans. The endotherm could therefore be due to dehydration. The water liberated on dehydration cannot escape from the volatile pans, and probably dissolves some of the dehydrated solid. Since the dissolution of the anhydrate in water is an endothermic reaction (see Fig. 9 and Table XI), the endotherm at ≃117°C will also include the heat of solution of the reaction(Scheme Ib). The pan now contains the anhydrate as well as an aqueous solution of the anhydrate. The presence of the latter seems to prevent the anhydrate from melting, due to some unknown reason.

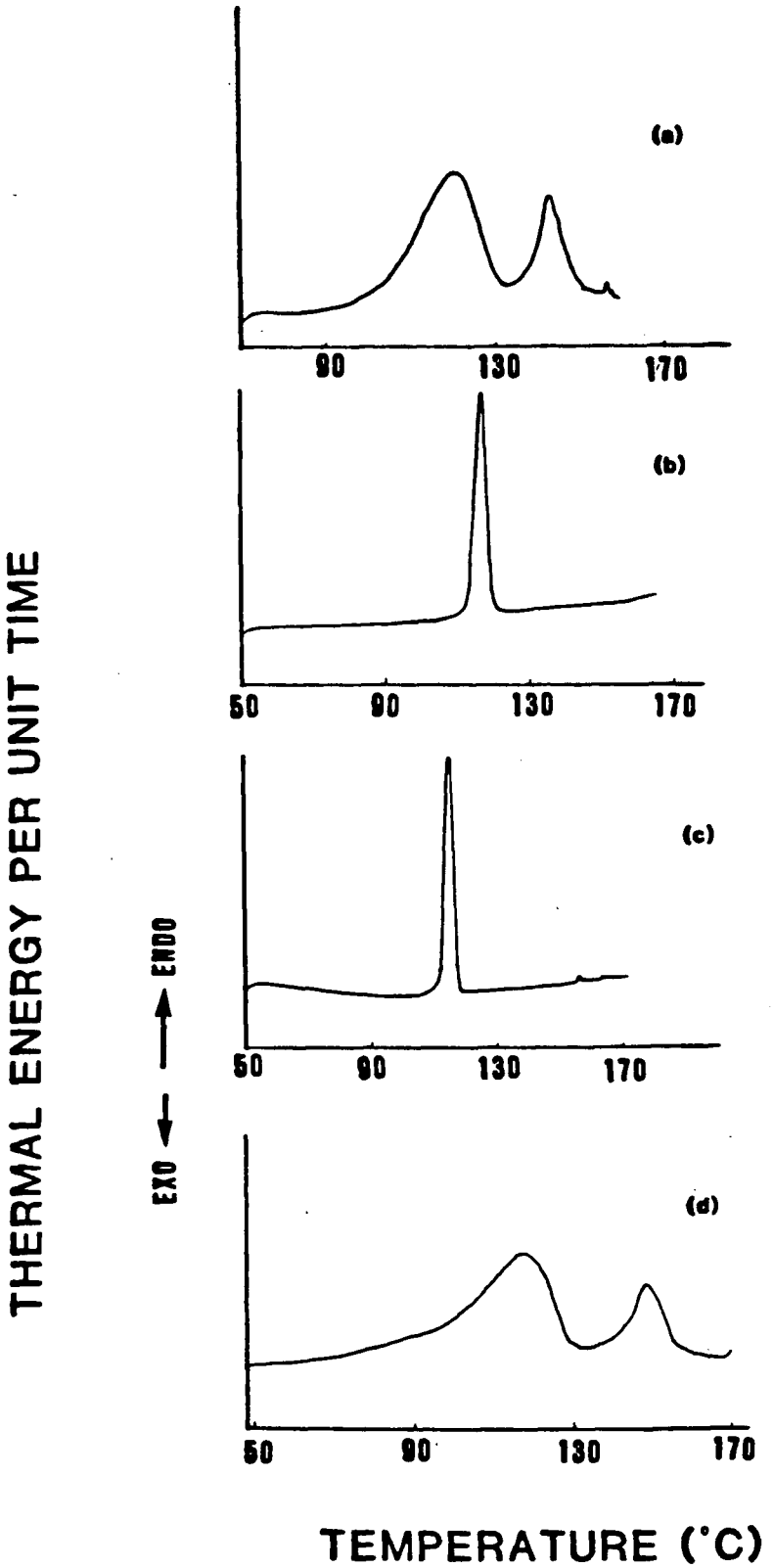

Fig. 11 Representative thermal curves of I (a) standard pan, (b) volatile pan, (c) volatile pan with a pinhole and (d) volatile pan with a pinhole under reduced pressure.

			peak temperature
a	standard	$CaGl \cdot 3\frac{1}{2}H_2O \longrightarrow CaGl(s) + 3\frac{1}{2}H_2O(g)$	119-122°C
		$CaGl(s) \longrightarrow CaGl(l)$	144-145°C
b	volatile pan	$CaGl \cdot 3\frac{1}{2}H_2O \longrightarrow CaGl(s) + CaGl(so) + H_2O(g)$	116-119°C
c	volatile pan with pinhole	$CaGl \cdot 3\frac{1}{2}H_2O \longrightarrow CaGl(s) + 3\frac{1}{2}H_2O(g)$	115-124°C
d	volatile pan with pinhole (under vacuum)	$CaGl \cdot 3\frac{1}{2}H_2O \longrightarrow CaGl(s) + 3\frac{1}{2}H_2O(g)$	107-118°C
		$CaGl(s) \longrightarrow CaGl(l)$	145-148°C

Scheme I Dehydration reactions and transitions of I in the differential scanning calorimeter; CaGl = calcium gluceptate, s = solid, g = gas, l = liquid and so = solution. The range of the peak temperatures is based on 3 or more DSC sample runs.

When I was heated in a volatile pan with a pinhole, there was an endothermic peak at ≃119°C accompanied by a weight loss of ≃11% w/w which suggests dehydration accompanied by the simultaneous vaporization of most of the dehydrated water (Fig. 11c, Scheme Ic). However, since no endotherm was observed at ≃145°C it seems likely that a small amount of the liberated water is adsorbed by the anhydrate and prevents melting. In volatile pans with a pinhole, the rate of water vapor loss becomes appreciable only when the internal pressure within the pan is equal to the atmospheric pressure. The restricted escape route (0.1 to 0.2 mm pinhole) could bring the water vapor and the dehydrated material into intimate contact, facilitating some adsorption of water by the anhydrate. It is not known how the adsorbed moisture prevents the melting of the anhydrate.

When a volatile pan with a pinhole was used under reduced pressure[1], the thermal behavior was similar to that seen with standard pans in that two endotherms occurred at ≃113°C and ≃147°C (Fig. 11d). Because of the reduced pressure, the water vapor released on dehydration (at ≃113°C) will be rapidly evaporated. Hence the anhydrate does not adsorb moisture and melts sharply at ≃147°C (Scheme Id).

I was also heated under atmospheric pressure in a volatile pan with a pinhole up to the baseline departure of

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[1]For these studies, a model 910 differential scanning calorimeter, Du Pont with a series 99 thermal analyzer was used.

the first endotherm at 115°C. The heating was then stopped and vacuum applied for 5 min. The run was continued under reduced pressure and a melting endotherm was observed at ≃145°C. This confirmed the role of adsorbed water in preventing the melting of the anhydrate since the application of vacuum at the dehydration endotherm will remove the water vapor before it can be adsorbed by the anhydrate.

In an attempt to quantitate the enthalpy of the transitions occurring on heating I, a DSC[2] with a data station[3] was used. In both standard pans and volatile pans with a pinhole, dehydration and vaporization occurred simultaneously and it was not possible to quantitate the enthalpy of dehydration. Moreover, in volatile pans with a pinhole, the loss of water following dehydration was incomplete possibly due to adsorption of some water vapor. The melting of the anhydrate, II, was accompanied by decomposition and so the enthalpy of melting was not determined.

When II was subjected to DSC immediately after preparation from I by dehydration, an endotherm occurred at ≃140°C, irrespective of the type of the pan used (Fig. 12a). There was no appreciable loss in the weight of the sample. Thermomicroscopy showed that this endotherm corresponded to melting. Since drying I at 60°C under vacuum for 16 h caused a weight loss of ≃11.3% w/w, II was identified as anhydrous calcium gluceptate. Drying for periods longer than 16 h did

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[2]Model DSC-2C, Perkin Elmer
[3]Model 3600, Perkin Elmer

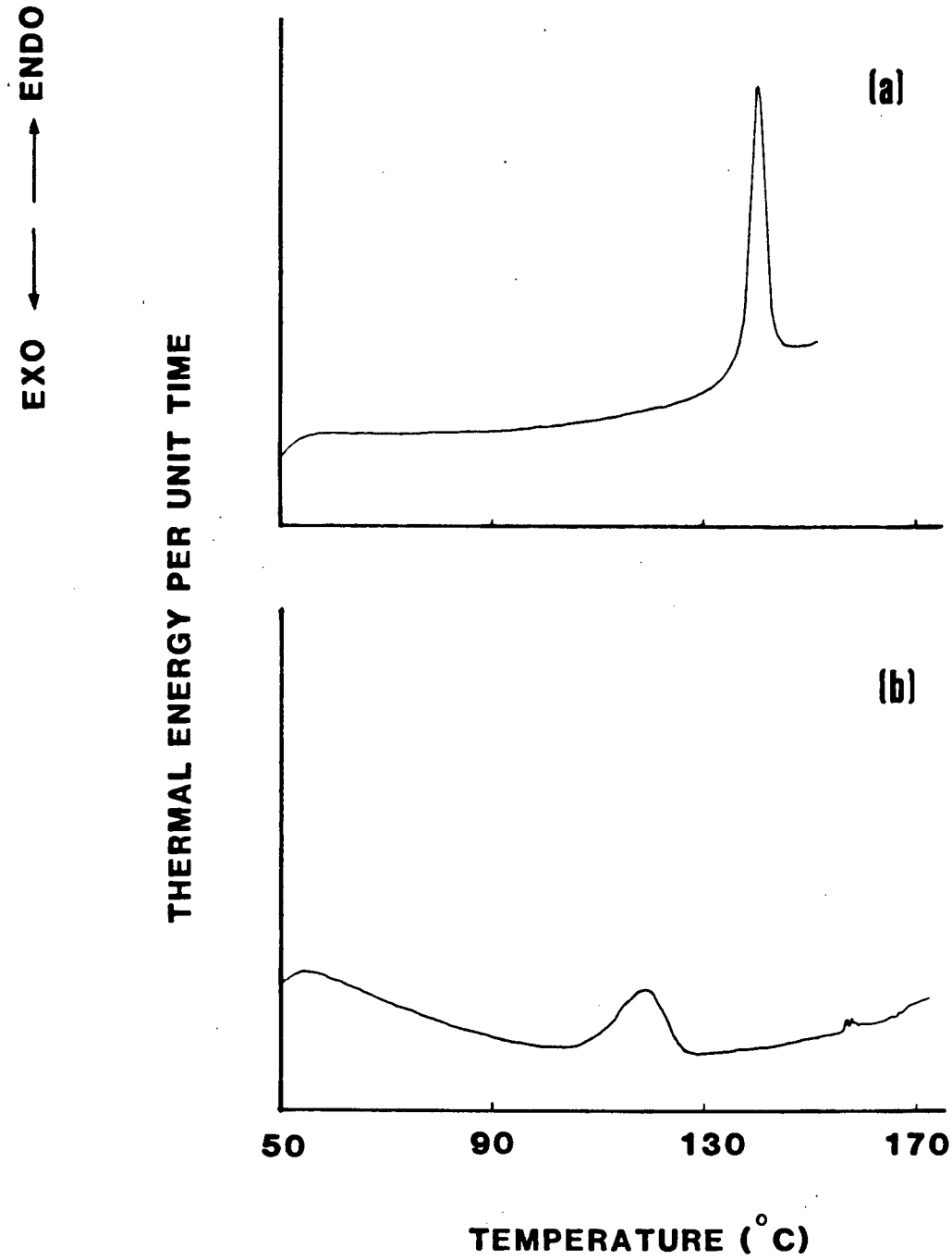


Fig. 12 Representative thermal curves of II in volatile pans (a) freshly prepared sample and (b) sample exposed to ambient conditions for 5 min.

not cause any further weight loss.

When II was exposed to ambient conditions (≃35% RH, 23°C) for 5 min, there was a small increase in weight (≃2% w/w) suggesting adsorption of moisture and on heating in volatile pans, there was a single endotherm at ≃118°C, Fig.12b. The increase in weight on exposure suggests adsorption of moisture which could lead to dissolution of some of the anhydrate on the surfaces of the particles. However, at room temperature, the anhydrate has a much higher apparent water solubility than the hydrate (1.3 molal compared with 0.07 molal, see Fig. 14 on p.88). Therefore the solution of the anhydrate would be supersaturated with respect to the hydrate and result in precipitation. The anhydrate would now have a surface layer of the hydrate and its dehydration could be responsible for the endotherm at ≃118°C. The water liberated at ≃118°C cannot escape from the volatile pans and probably redissolves some of the anhydrate. The anhydrate peak at ≃118°C will also include the heat of solution of this reaction. It is hypothesized that the presence of an aqueous solution of anhydrate prevents the melting of the anhydrate.

5. Equilibrium solubility

Table VII contains the equilibrium solubilities of I determined at different temperatures. A van't Hoff plot of log solubility against 1/T where T is the temperature in °K resulted in the linear relationship shown in Fig. 13. The heat

Table VII Equilibrium solubility of I at different temperatures.

Temperature (°C)	Solubility[a] (molal)
25.5	0.0581 ± 0.0006[b]
31.5	0.0791 ± 0.0010[b]
34.0	0.0922 ± 0.0003[b]
37.0	0.1048 ± 0.0008[c]

[a]Mean ± S.D.; [b]n=6; [c]n=3.

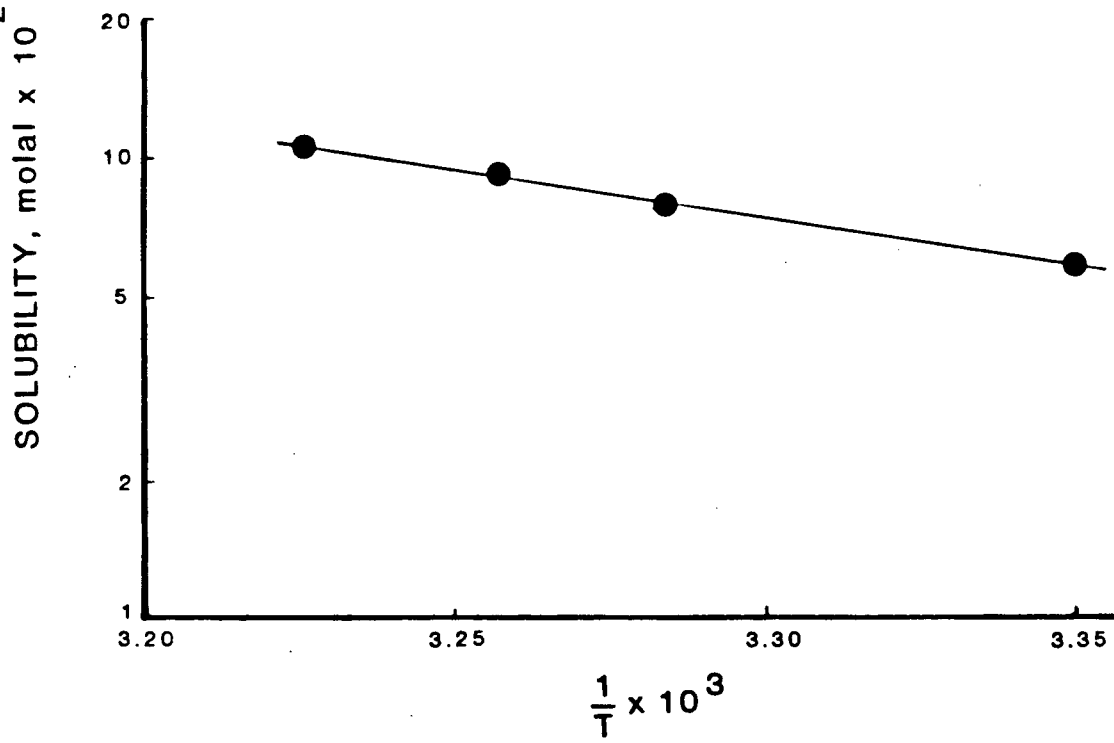


Fig. 13 The van't Hoff plot for I in water.

of solution of I calculated from this plot was 40.0 kJ mol^{-1}. Grant *et al.* (1984) have recently shown that van't Hoff plots could be non-linear particularly when determined over a wide temperature range of 50 degrees or more. Hence the solubilities of I were determined over a narrow temperature range (11.5°C) and the van't Hoff plot was assumed to be linear.

Attempts to determine the maximum solubility of II were unsuccessful. It had a high initial solubility at room temperature (≃22°C) but the solutions were unstable resulting in rapid precipitation. The powder x-ray diffraction pattern of this precipitate was identical to that of I (see Table VI on p. 72) . Suryanarayanan and Mitchell (1984) observed that on dissolution in water, amorphous anhydrous calcium gluceptate precipitated as the crystalline hydrate. A similar solution phase transformation from an anhydrate to a hydrate has been observed in several compounds including cholesterol, theophylline, glutethimide (Shefter and Higuchi, 1963) and mercaptopurine (Huang and Niazi, 1977). In several instances, the equilibrium solubility of the hydrate and the apparent solubility of the corresponding anhydrate have been determined. For example, in the case of theophylline, dehydration of the monohydrate caused a 1.8-fold increase in apparent aqueous solubility at 35°C (Shefter and Higuchi, 1963). The apparent aqueous solubility at 37°C, of anhydrous mercaptopurine was calculated to be 1.6 times that of the equilibrium solubility of mercaptopurine monohydrate (Huang

and Niazi, 1977; Niazi, 1978). The apparent solubility of II at ≃22°C was found to be 1.29 molal while that of I was 0.07 molal (Fig. 14). Thus dehydration of calcium gluceptate hydrate produces an 18-fold increase in its apparent solubility. We are not aware of any other hydrate that undergoes such a dramatic increase in its apparent water solubility on dehydration.

6. Characterization of different samples of calcium gluceptate

Calcium gluceptate obtained from different sources was characterized (Table VIII). The relative proportions of the α and β epimers were determined by the GC method (section C.1 in Experimental). According to the USP XX (1980c), calcium gluceptate can be an anhydrate or a hydrate with either 2 molecules (6.9% w/w water) or 3 1/2 molecules (11.4% w/w water) of water of crystallization. Irrespective of the commercial source, all samples were either an amorphous anhydrate or a crystalline hydrate with 3 1/2 molecules of water of crystallization (Table VIII). In the latter case, the weight loss on heating in the DSC (in both standard pans and volatile pans with a pinhole) was ≃11% w/w.

All the amorphous, anhydrous samples were manufactured prior to July, 1980 with the exception of the material

Table VIII. Some solid-state properties of various samples of calcium gluceptate.

Sample	Date of Manufacture	Relative proportions of α and β epimers	Powder x-ray pattern	No. of molecules of water of crystallization	water solubility
Pfanstiehl; lot 7311[a]	1966	52%α;48%β	A	0	very soluble[b]
Sigma; lot 126C-0121	1976[c]	100%α	A	0	very soluble[b]
Givaudan; lot R 3679 BA[a]	1979[c]	72%α;28%β	A	0	very soluble[b]
Italsintex; lot R 1432 TJ[a]	1979[c]	72%α;28%β	A	0	very soluble[b]
Pfanstiehl; lot 12953-D(III)[a]	Jan. 1980	100%α	A	0	very soluble[b]
Pfanstiehl; lot 13313-E(I)	July 1980	100%α	C	3 1/2	sparingly soluble[d]
Pfanstiehl; lot 14772	Blend of materials made in Dec. 1980 and Mar. 1982	100%α	C	3 1/2	sparingly soluble[d]
Pfaltz and Bauer; lot C01300	1982[c]	100%α	C	3 1/2	sparingly soluble[d]
PMH	1982[c]	100%α	C	3 1/2	sparingly soluble[d]
Merck, ST-16944; RM-45940	1984	100%α	A	0	very soluble[b]

[a] Samples used in earlier investigations (Suryanarayanan and Mitchell, 1984).

[b] Equilibrium solubility can not be determined; apparent solublity >100% w/v at room temperature (approx. 22°C) for all amorphous samples; 27% w/v solutions of all the samples (except Pfanstiehl; lot 7311) precipitated on storage.

[c] Year obtained; actual manufacturing date not known.

[d] Equilibrium solublilty at 25.5°C was 3.2% w/v; all crystalline samples had similar solubility values.

A = amorphous to x-rays. C = crystalline; all samples had identical x-ray diffraction patterns.

manufactured by Merck Frosst Canada in 1984. Calcium gluceptate manufactured from July, 1980 onwards was a crystalline hydrate irrespective of the commercial source (again the material from Merck Frosst Canada was an exception). Holstein in 1980 had observed that at Pfanstiehl Laboratories, preparation of calcium gluceptate in the amorphous form had become impossible due to the presence of seed crystals (presumably of calcium gluceptate hydrate) or some other factor initiating crystallization of calcium gluceptate hydrate. It appears that a similar problem was encountered by other manufacturers of calcium gluceptate with the curious exception of Merck Frosst Canada.

B. TREATMENT OF CALCIUM GLUCEPTATE

1. Grinding

I ground for 1 h and II ground for 4 h were subjected to GC analysis (see section C.1 in Experimental). Both the samples contained a single compound with a retention time of 9.2 min. This compound was earlier identified to be calcium *a*-glucoheptonate (section A.1 in Results and Discussion). The absence of additional peaks proved that grinding I and II for 1 and 4 h respectively caused no detectable sample decomposition.

1.1 Effect on apparent solubility

The effect of grinding time on the solubility of I and II is shown in Fig.14. Grinding had a marked effect only on the apparent solubility of II. The apparent solubility of unground II was 1.3 molal while that of II ground for 2 h was 5.4 molal i.e. a 4.2-fold increase in apparent solubility. Table IX lists the effect of grinding on the dissolution behavior of some other drugs. In one sample of digoxin, grinding caused an ≃2.2-fold (118%) increase in apparent solubility. Thus the apparent solubility of II is extremely sensitive to grinding time. Attempts to determine the apparent solubility of II ground for longer than 2 h were unsuccessful because of the extreme instability of these solutions which caused a very rapid precipitation of I from solution.

1.2 Effect on surface area

Only the surface areas of unground and ground II were determined (Table XI, p. 106). Even after grinding for 4 h there was less than a 3-fold increase in surface area.

1.3 Effect on x-ray diffraction

The powder x-ray diffraction patterns of I ground for different times shown in Fig. 15 suggest that grinding for 1 h, makes it x-ray amorphous. To produce a similar effect in II, a longer grinding time of 4 h was required (Fig. 6). Scheme II contains the apparent solubilities as well as the

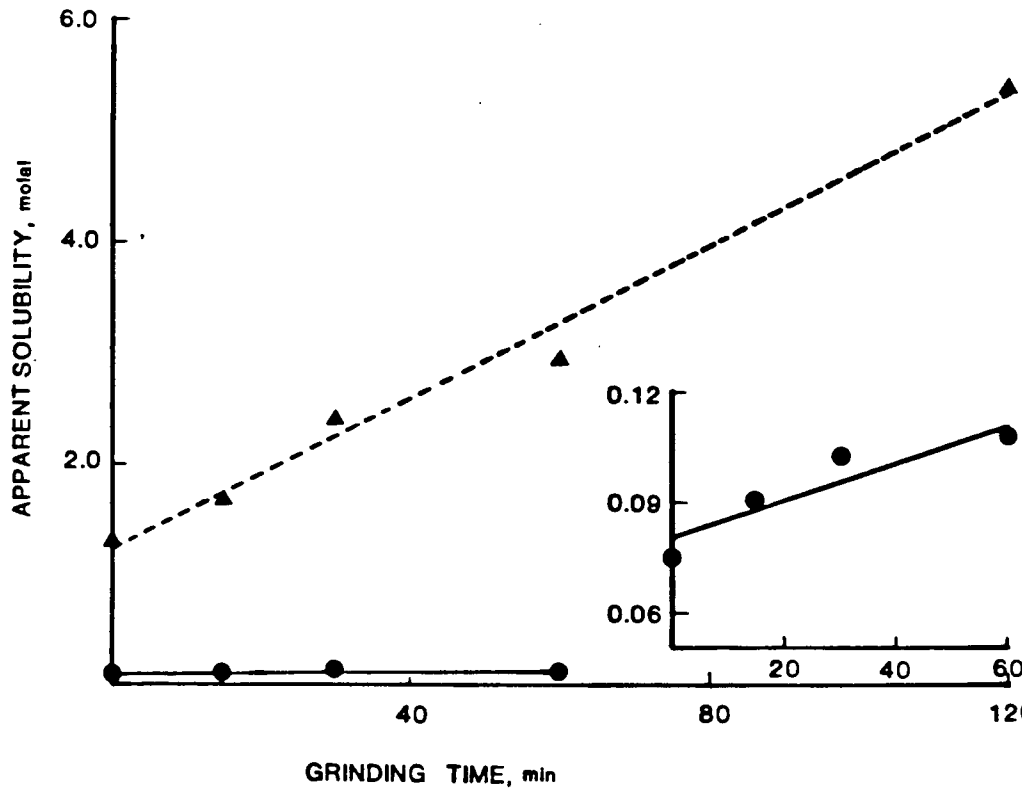


Fig. 14 Effect of grinding on the apparent solubility of I (—●—●—) and II (·▲----▲--) in water at room temperature (≈22°C). Inset: Apparent solubility values of I plotted on an expanded scale. All values are averages of two determinations.

Table IX Effect of grinding on the dissolution behavior of some drugs.

Drug	Type of grinder	Grinding time	Effect (in comparison with unground drug)	Reference
Digoxin	Mortar and pestle	Not known	In simulated gastric fluid, 66% of the digoxin dissolved in 2 h compared to the dissolution of 52% of unground digoxin[a]	Shah et al., 1974
Digoxin from different sources	Ball mill	3½-8 h	Apparent aqueous solubilities increased between 7 and 118%	Florence and Salole, 1976
Griseofulvin	Ball mill	24 h	No increase in dissolution rate (in Disintegration Medium No.1 of Japanese Pharmacopeia X)	Sawayanagi et al., 1982
Cephalexin	Shaker mill	4 h	Approximately 100% increase in apparent equilibrium solubility in water	Otsuka and Kaneniwa, 1984

[a] These results may be unreliable because of the rapid degradation of digoxin from such highly acidic dissolution media (Sonobe et al., 1980).

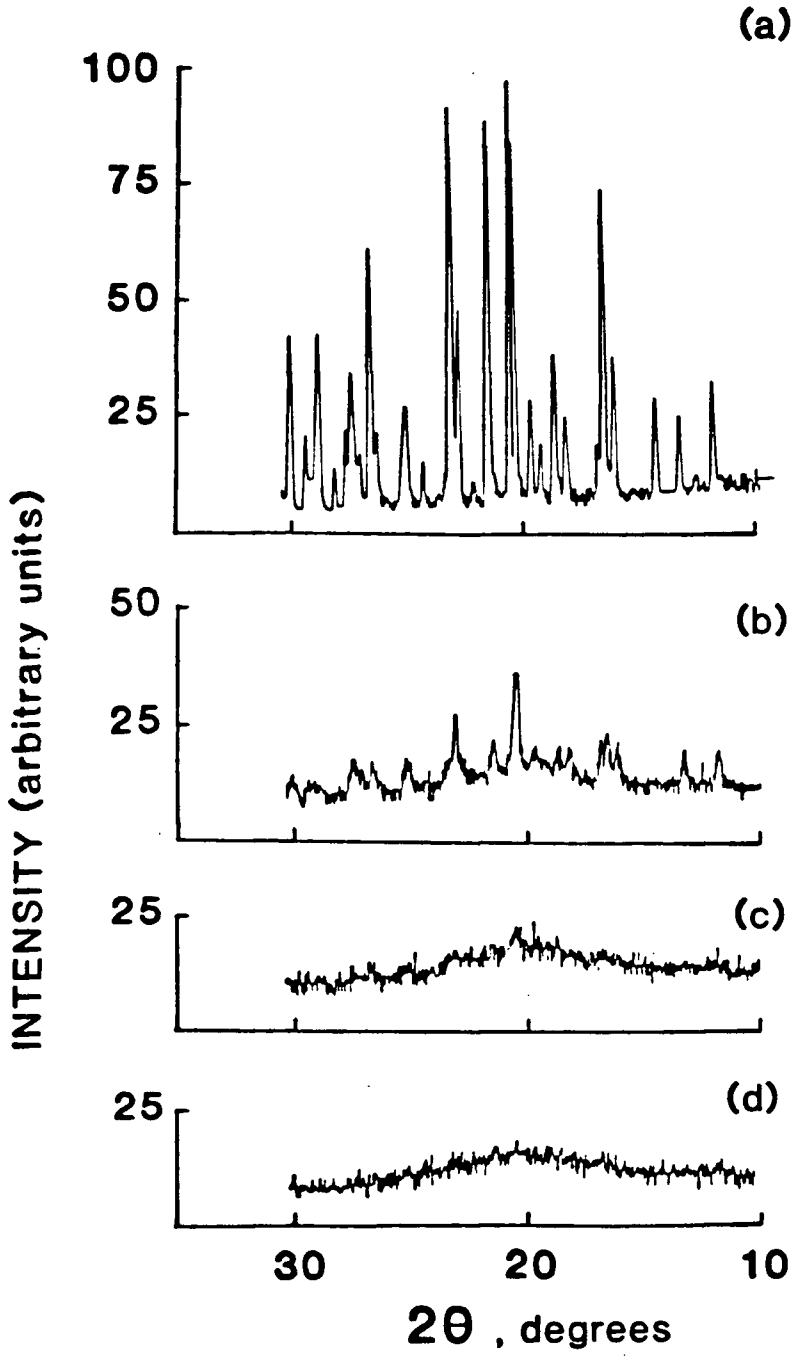


Fig. 15 Powder x-ray diffraction patterns of I (a) unground; (b), (c) and (d) ground for 15, 30 and 60 min, respectively.

method of preparation of some solid phases of calcium gluceptate.

2. Freeze drying

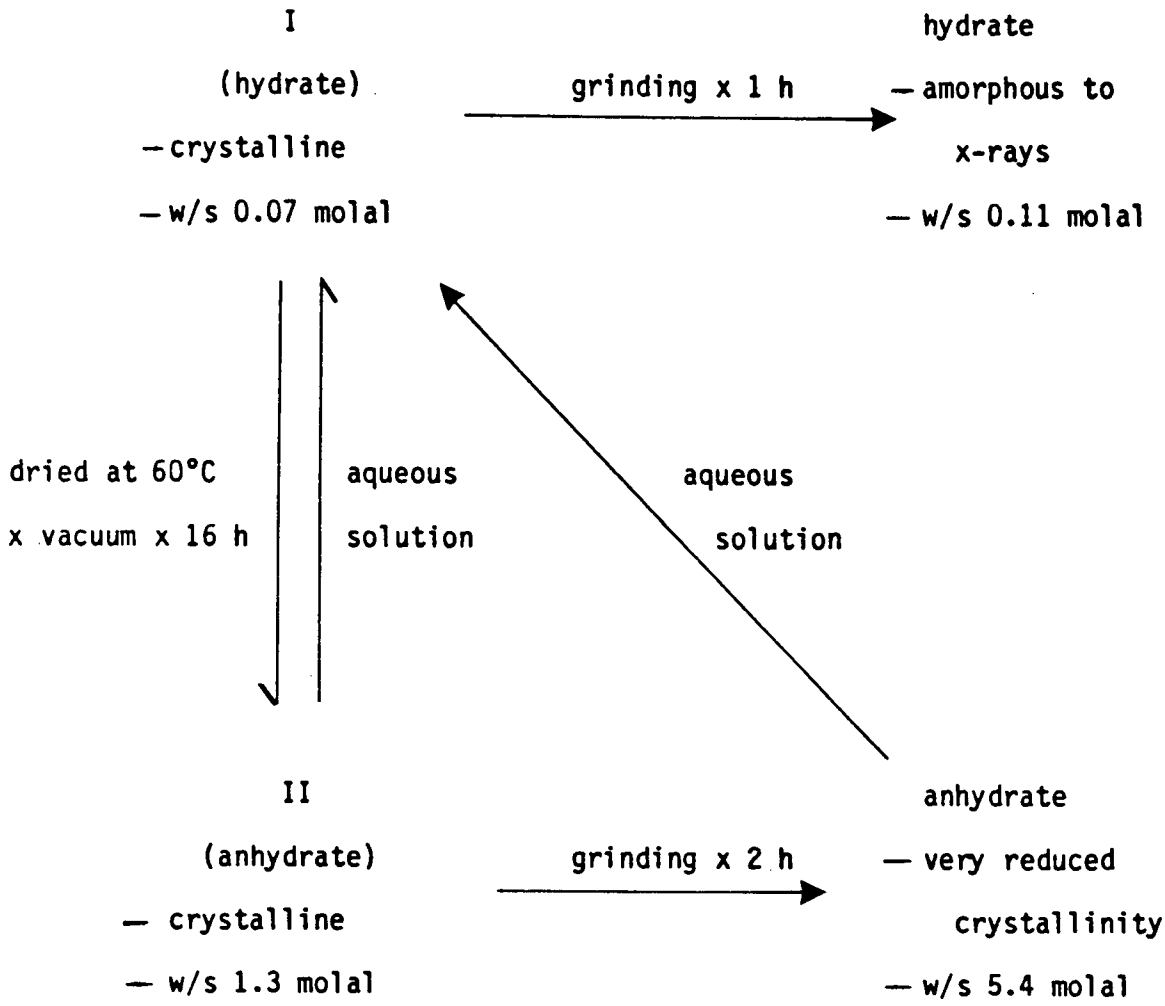
The product obtained on freeze drying an aqueous solution of I had the following properties:

1. it did not diffract x-rays and was therefore x-ray amorphous.
2. the DSC thermogram, showed no endothermic or exothermic peaks between 30 and 180°C indicating that it was anhydrous and also that there are no phase transitions in this temperature range. The absence of a melting point was also indicative of its non-crystalline nature (Ke, 1966).
3. GC analysis (see section C.1 in Experimental) resulted in the elution of a single compound at 9.2 min which is due to calcium *a*-glucoheptonate.

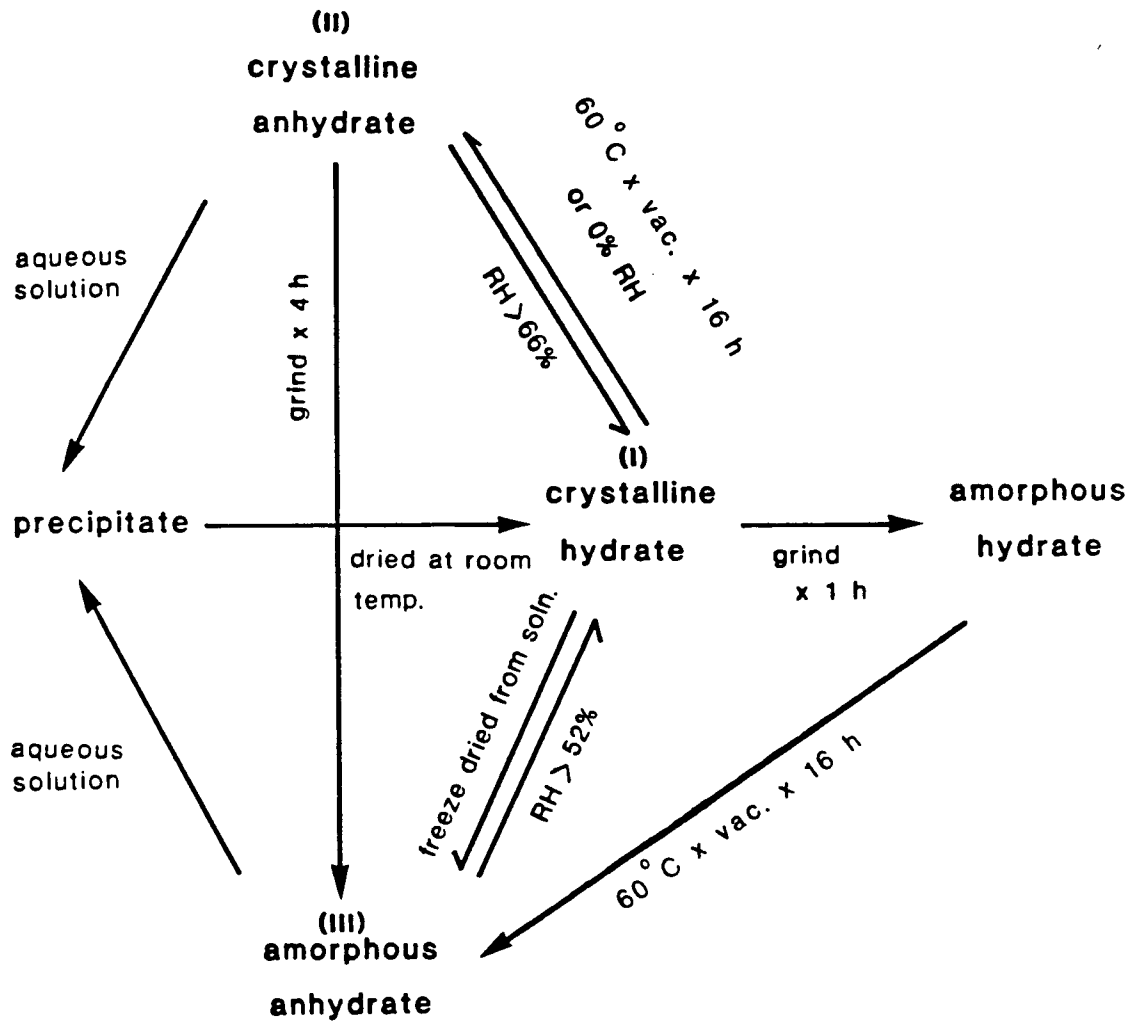
Hence the freeze dried product was amorphous anhydrous calcium gluceptate.

3. Constant humidity studies

Storing I, II and III at a range of relative humidities resulted in the phase transitions shown in Scheme III. While the transition of the crystalline anhydrate (II) to the crystalline hydrate (I) occurred at RH > 66%, the transition of the amorphous anhydrate (III) to I occurred at RH > 52%,



Scheme II. Some phases of calcium gluceptate and their aqueous apparent solubilities (w/s = apparent water solubility), at room temperature (approx. 22°C)



Scheme III Phase transitions of calcium gluceptate.

showing that the more energetic amorphous form required a lower relative humidity for the transition. Huttenrauch (1977a) observed that the hygroscopicity of sucrose was related to its crystallinity. While the moisture adsorption of amorphous sucrose increased exponentially with increasing RH starting at 50% RH, crystalline sucrose did not adsorb moisture up to 70% RH.

The transition of I to II and vice versa gave some interesting results (Fig. 16). I could be dehydrated either by the USP method (drying at 60°C under vacuum for 16 h) or by storage at 0% RH. However, the anhydrous form, II, did not undergo a transition to the hydrate, I, until the RH was raised above 66%. Between 0% and 66% RH both I and II seemed to be stable and neither underwent a phase transition. This observation is contrary to the phase rule according to which both the anhydrous form and the hydrate of a compound cannot be stable at one temperature over a range of vapor pressures. Therefore in the 0% to 66% RH range, the rate of conversion of the anhydrate to hydrate or vice versa must be a very slow process.

Mitrevej and Hollenbeck (1983) showed the existence of a hydrophobic field generated by aspirin crystals which inhibited the condensation of water from an high humidity environment in the vicinity of the crystals. When aspirin was mixed with certain hydrophilic excipients such as colloidal silicon dioxide, water readily condensed on the crystals. It

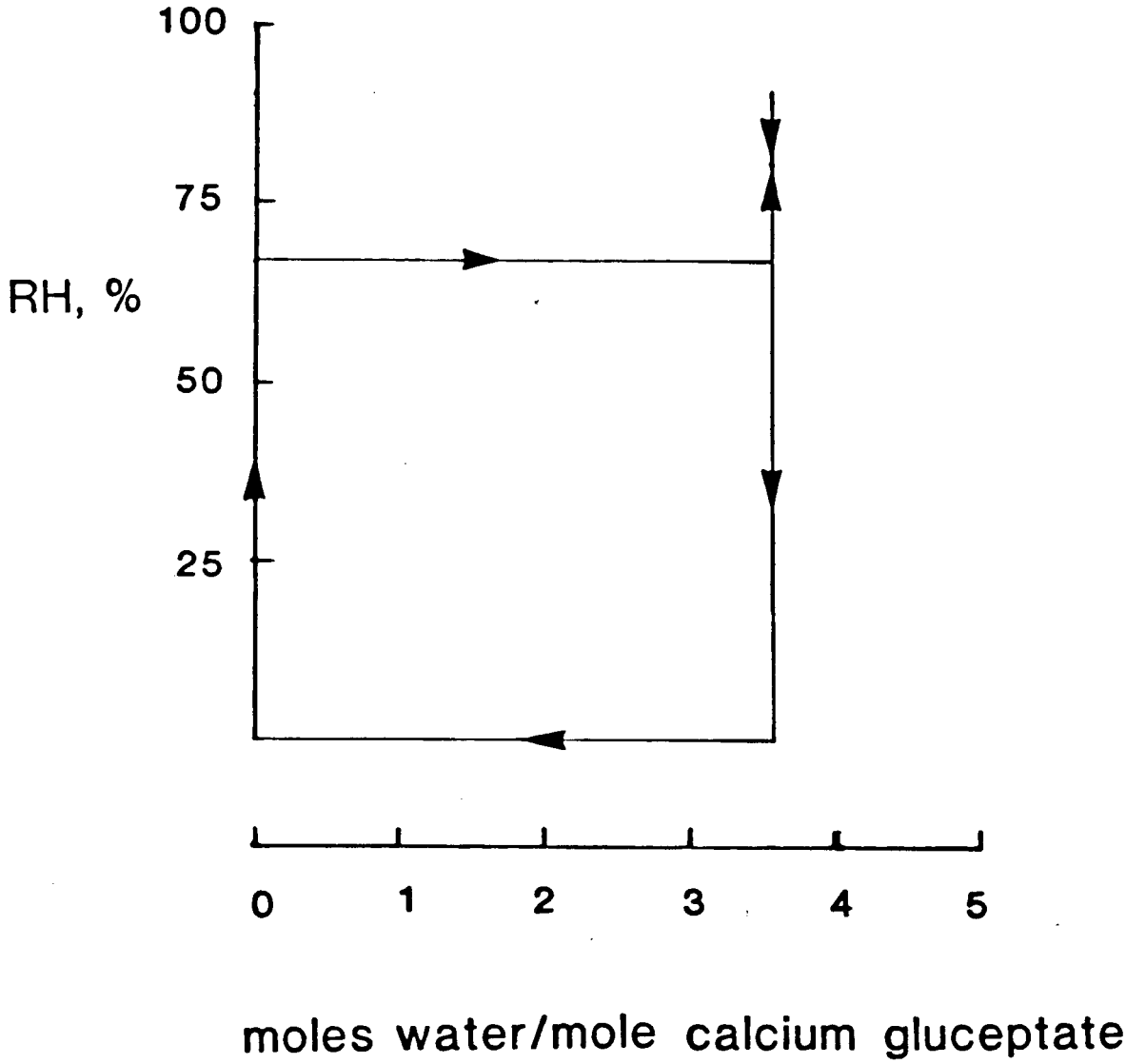


Fig. 16 The hydrate(I) - anhydrate(II) transitions of calcium gluceptate at 25°C.

is possible that crystals of II may generate a similar field. This could explain its stability up to 66% RH, while if, at higher RH values, the water vapor pressure successfully overcomes this field the transition from II to I could occur. In order to test this hypothesis, II was mixed with 1% and 5%w/w colloidal silicon dioxide and stored at various humidities. The transition from II to I continued to occur only above 66% RH indicating that II did not generate a hydrophobic field.

Another possibility is that of an energy barrier to the dehydration (I to II) and rehydration (II to I) reactions. To investigate this, the energy of activation, E_a, for the dehydration of I to II was calculated using the following relationship(Kissinger, 1957):

$$\frac{d(\ln \phi/T_m^2)}{d(1/T_m)} = -\frac{E_a}{R} \qquad (9)$$

where ϕ is the heating rate (°C min^{-1}), T_m is the temperature (°K) at which peak enthalpic deflection occurs and R is the gas constant. A plot of $\ln \phi/T_m^2$ versus $1/T_m$ gives the activation energy without any assumptions about the reaction mechanism. Samples of I were heated at 2.5, 5, 10, 20 and 40 °C min^{-1} in a DSC and the temperature of peak enthalpic deflection (the peak due to dehydration and vaporization of water at ≃121°C, Scheme Ia) was noted.

The energy of activation for dehydration of I calculated from the Kissinger plot was 92.5 kJ mol^{-1}(Fig. 17). Table X

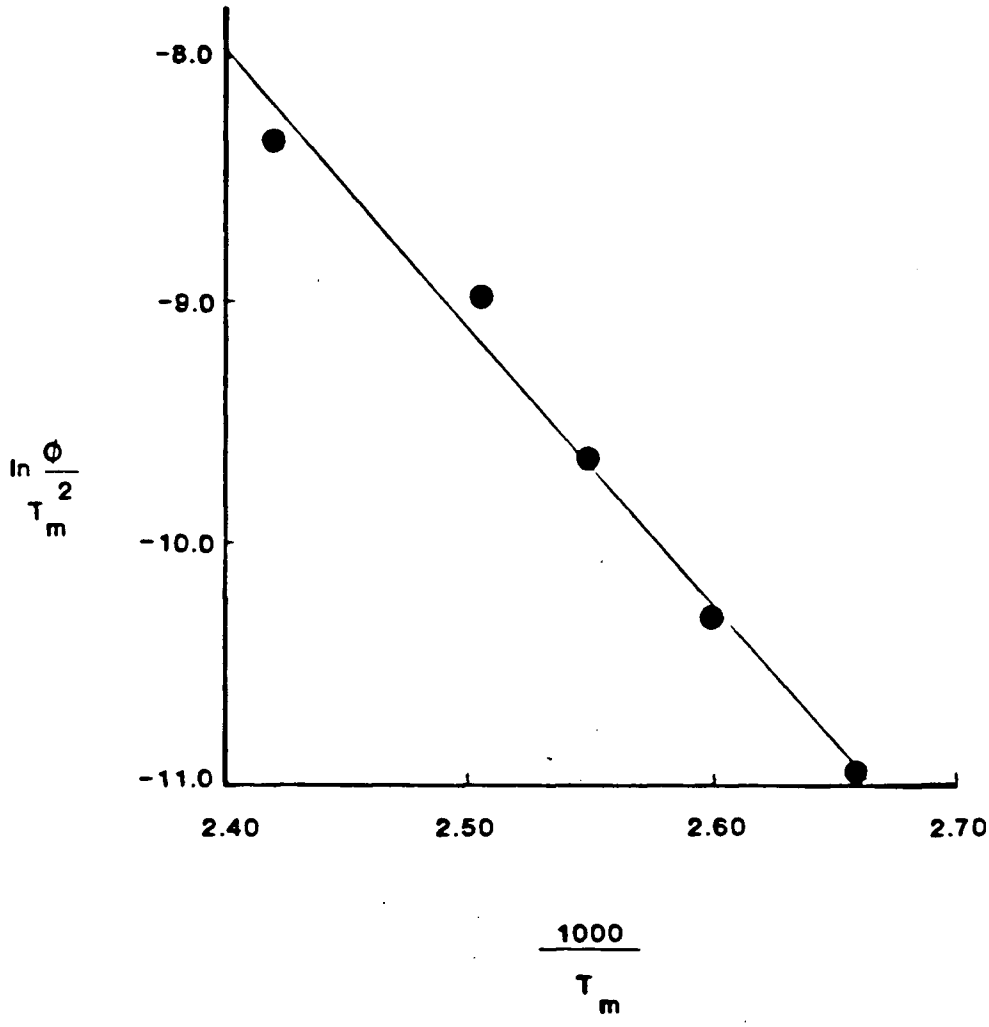


Fig. 17 Kissinger plot for calculation of the activation energy for dehydration of I [T_m=temperature of peak (°K); ϕ=heating rate (°C min^{-1})].

lists the energy of activation for dehydration of some pharmaceutical hydrates. The energy of activation for dehydration of I is not unusually high when compared with the values listed in Table X and suggests that an energy barrier for the dehydration of I to II is unlikely.

The energy of activation for the transition of II to I was also determined. The heat of solution of I calculated from the van't Hoff plot was +40.0 kJ mol^{-1}(section A.5 in Results and Discussion, p.80) and that of II determined by solution calorimetry was +13.8 kJ mol^{-1}(Table XI, p.106). The difference in the heats of solution, ΔH_d, is the heat change accompanying the dehydration reaction and has a value of +26.2 kJ mol^{-1}(Scheme IV). The energy of activation for dehydration of I, E_a, calculated from the Kissinger plot was 92.5 kJ mol^{-1}. Since the change from I to II is a reversible process, the energy of activation for the transition of II to I, E_b, can be calculated from (Glasstone and Lewis, 1982b):

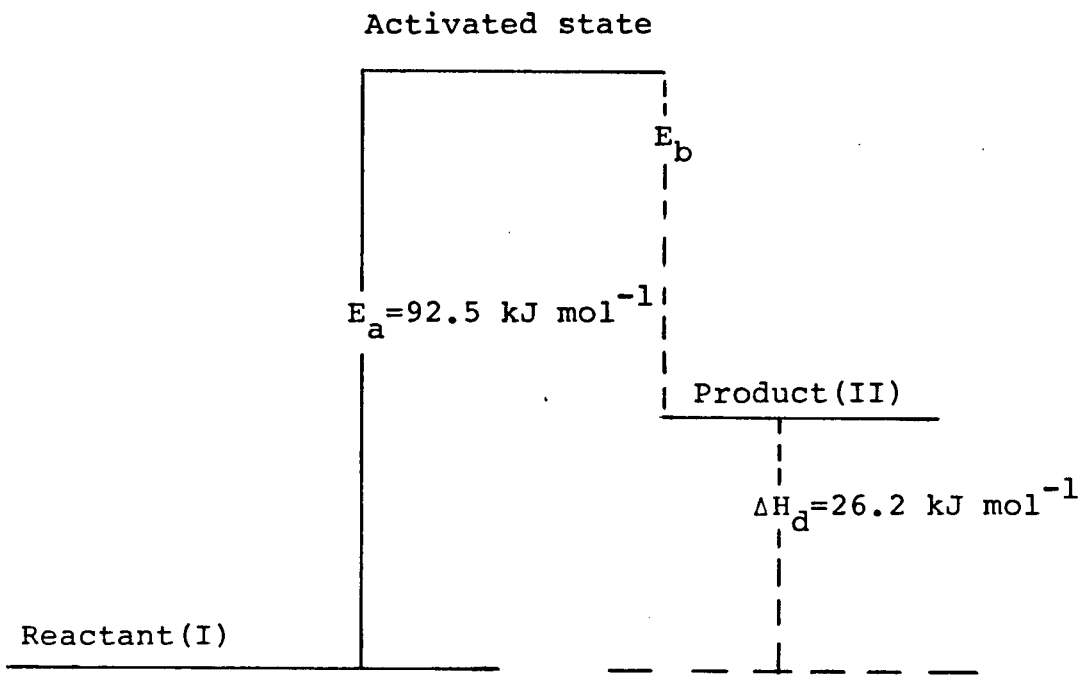
$$E_a - E_b = \Delta H_d \qquad (10)$$

E_b was calculated to be 66.3 kJ mol^{-1} and from the low value of E_b, an energy barrier for transition of II to I seems unlikely.

The desolvation of certain hydrates may be initiated by inoculation with the desolvated material, and this has been demonstrated in cytosine hydrate (Byrn, 1982). If it were similarly possible to initiate the dehydration of I, the

Table X Activation energies for some dehydration reactions.

Compound	Energy of activation ($kJ\ mol^{-1}$)	Reference
Cefamandole sodium monohydrate → anhydrate	71	Pikal et al., 1983
Ampicillin monohydrate → anhydrate	95	Shefter et al., 1973
Sodium prasterone sulfate dihydrate → anhydrate	131	Nakagawa et al., 1981
Theophylline monohydrate → anhydrate	140	Shefter et al., 1973
Sulfaguanidine monohydrate → anhydrate	Between 67 and 168 depending on crystallinity of the samples as well as environmental factors	Sekiguchi et al., 1984
Mercaptopurine monohydrate → anhydrate	Between 191 and 264 depending on the method of determination	Niazi, 1978



Scheme IV Some thermodynamic values of dehydration of I to II. These values were used to calculate the energy of activation, E_b, for transition of II to I.

reaction may proceed at RH values higher than 0%. To test this, samples of I were initially stored at 0% RH. As soon as they began to dehydrate to II (which was evident from the samples losing weight), they were transferred to chambers at 9, 33 and 52% RH. The samples did not continue to lose weight, so both I and II were capable of coexisting at these humidities. Similarly, the conversion of II to I by inoculation with I was also attempted. Samples of II were initially stored at 79% RH and when they began to gain weight and change to I, they were transferred to chambers at 9, 33 and 52% RH. The samples did not continue to gain weight, again confirming that II and I could coexist at these humidities.

Though samples of II stored in the range of 0-66% RH did not undergo a transition to I, samples stored above 0% RH gained weight due to the adsorption of moisture. The increase in weight ranged from 0.84% w/w at 9% RH to 3.3% w/w at 66% RH. If the adsorbed moisture dissolves some of the anhydrate, the solution would be supersaturated with respect to the stable hydrate and could result in precipitation of the hydrate on the surface of the anhydrous crystals. It is suggested that this hydrated layer acts as a barrier to the diffusion of water vapor and thus prevents the conversion of II to I up to 66% RH. Earlier work with DSC (section A.4 in Results and Discussion) showed how the thermal behavior of II was affected by the adsorption of a small amount of moisture. When II was stored at RH > 66%, the water vapor pressure was presumably high enough to overcome the resistance of the

hydrated surface layer to the penetration of water vapor and transition to I occurred (Fig.16).

C. STABILIZATION OF CALCIUM GLUCEPTATE SOLUTIONS

Aqueous solutions containing between 20 and 27% w/v II were prepared. Some solutions were left unfiltered while others were filtered either through a filter paper (Whatman No. 1)or a membrane filter (0.22μm). Immediately following this, the solutions were autoclaved at 121°C for 20 min. All the solutions were stable showing that neither the concentration of II nor the filtration procedure affected solution stability. The unfiltered solutions contained some undissolved solid, but this did not induce nucleation and crystallization in the autoclaved solutions. Suryanarayanan and Mitchell (1984) hypothesised that the precipitation of calcium gluceptate solutions was induced by seed crystals of calcium gluceptate hydrate and that autoclaving destroyed these seed crystals. These results support this hypothesis. When one sample was exposed to filtered air in a laminar flow cabinet or in a "clean" room which housed the laminar flow cabinet, it continued to be stable. When the same solution was exposed to the atmosphere in the "Pharmaceutics Laboratory", precipitation started within 24 h. All the previous experimental work with calcium gluceptate was done in the Pharmaceutics Laboratory and the precipitation was presumably due to the nucleation by seed crystals dispersed throughout this laboratory. The "clean" room is in relatively close

proximity to the Pharmaceutics Laboratory and some of the seed crystals are likely to drift to this room. It is suggested that the high efficiency particulate air filter (HEPA filter) in the laminar air flow cabinet effectively removes the seed crystals from the air both within the hood and the "clean" room. It therefore seems that autoclaved solutions of II will be stable when exposed to air as long as there are no seed crystals of calcium gluceptate hydrate in the atmosphere.

Calcium gluceptate USP is commercially available as a hydrate with a very low equilibrium water solubility of ≃3% w/v. However, 'stable' (no precipitation during two years of storage) solutions with a high calcium gluceptate concentration (≃25% w/v) can be prepared from this material. Dehydration of the commercial material dramatically increases its apparent water solubility and aqueous solutions of the required concentration can be prepared with the anhydrate, provided, the solutions are autoclaved immediately after preparation. This method is suitable for the preparation of sealed parenteral formulations but probably not for multi-dose oral preparations where exposure to the atmosphere may lead to seeding and recrystallization.

D. DETERMINATION OF DEGREE OF CRYSTALLINITY OF CALCIUM GLUCEPTATE

The degree of crystallinity determinations were confined to ground II because of the significant effect of grinding on

its apparent water solubility.

1. Selection of crytalline and amorphous reference standards

II was produced by drying I at 60°C under vacuum for 16 h and this was used as the crystalline (100% crystallinity) reference standard. When it was ground for 4 h, it became amorphous to x-rays (Fig. 6). Examination by polarized light microscopy revealed that unground II consisted of long thin crystals which were highly birefringent. The size of the 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 most of the particles were non-birefringent. It is apparent that polarized light microscopy was a more sensitive indicator of crystallinity than x-ray diffraction, since some particles still showed birefringence. Nakamachi *et al.* (1981) dehydrated mercaptopurine monohydrate and also obtained an x-ray amorphous intermediate which exhibited birefringence when examined microscopically under polarized light. Since the particles are either birefringent or non-birefringent, polarized light microscopy only gives a qualitative indication of the progressive decrease in crystallinity with grinding. On the other hand, decreasing crystallinity was readily quantitated from powder x-ray diffraction patterns from the gradual decrease in the intensity of the diffraction peaks.

With increasing grinding time, the DSC of II (in standard pans) showed a progressive decrease in the area of its melting endotherm at ≃144°C (DSC of unground II is discussed in section A.4 of Results and Discussion). The sample ground for 4 h did not melt at all and the absence of a sharp melting point is a characterestic feature of non-crystalline materials (Ke, 1966). Based on x-ray and DSC studies, II ground for 4 h was selected as the 0% crystalline (amorphous) standard.

2. Comparison of crystallinity values obtained by different methods

The crystallinity of II decreased with grinding time and Table XI lists the percent crystallinity values obtained by different methods. The values are in poor agreement.

In the pharmaceutical literature, the degree of crystallinity has usually been calculated assuming 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 Eq. 2 (see section C.1 in Introduction, p.9) and calorimetric crystallinity according to Eq. 4 (see section C.2.1 in Introduction, p.12). 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 reference standards (Fig. 7 and 9). Eq. 2 and 4 are based on a two-state model of crystallinity of polymers according to

Table XI Effect of grinding on some properties of II.

Grinding time (min)	Apparent solubility (molal)	Surface area ($m^2\ g^{-1}$)	Density ($g\ cm^{-3}$)	Particle diameter (assuming spherical particles) [nm(Å)]	Heat of solution ($kJ\ mol^{-1}$)	% Crystallinity by x-ray	% Crystallinity by heat of solution	% Crystallinity by density
0	1.29	5.45	1.6621	662(6620)	+13.77	100[a]	100[a]	100[a]
15	1.67	8.16	1.6720	440(4400)	+5.91	72.4	61.8	68.3
30	2.41	8.05	1.6782	438(4380)	-1.46	32.2	31.3	48.4
60	2.96	10.51	1.6822	340(3400)	-6.09	24.8	12.2	35.6
240	ND	14.08	1.6933	250(2500)	-9.13	0.0[a]	0.0[a]	0.0[a]

[a] assumed

ND = not determined

which, small but perfect crystalline regions are embedded within a continous amorphous matrix (for more details see section B.1 in Introduction, p.3). However, even in polymers, such a model is recognized as a gross oversimplification 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 XI 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. 2, 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 earlier, 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 paticle 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 the 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. Hence, formation of an amorphous layer surrounding a crystalline core does not describe the decreasing crystallinity of II as grinding continues. Rather than the surface layers of a particle becoming completely amorphous, it is probable that grinding causes more disorder in the surface layers than in the bulk, and that the disorder progressively decreases towards the core of each particle.

The results from polarized light microscopy can also be used to refute the two-state model. 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 XI).

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 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 (Appendix II), assuming that all the particles were spherical in shape and of uniform size (Table XI). The effect of particle size on x-ray line breadth usually becomes apparent only when the crystal size is below 100 nm(Cullity, 1978c). Since after 4 h grinding, the hypothetical particle diameter of II was about 250 nm (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 mol^{-1} (from -198.19 kJ mol^{-1} to -198.75 kJ 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 XI 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 relationship between degree of crystallinity and apparent solubility is shown in Fig. 18. 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 $\simeq 2.5 \times 10^{5}$ 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.

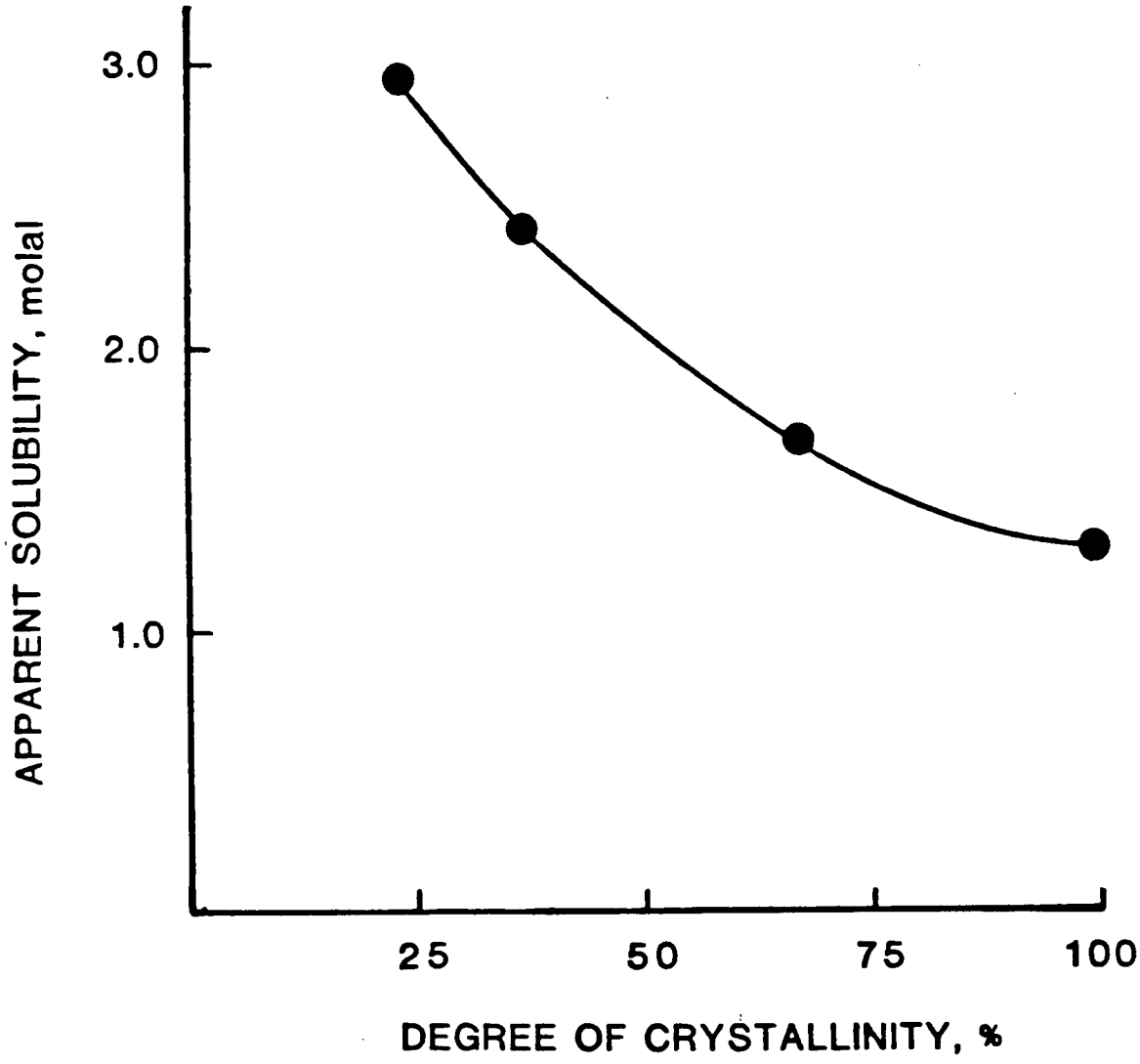


Fig. 18 Relationship between the degree of crystallinity and apparent solubility of II in water at room temperature (≈22°C). The individual crystallinity values are averages of the values determined by x-ray, calorimetry and density for each grinding time (Table XI).

In addition to the creation of lattice disorder, grinding may increase 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 greater than could be accounted by the 2.0-fold reduction in hypothetical particle size (see Appendix III for calculations). Since unground II occurred as long thin crystals, the surfaces created on fracture may be more reactive than surfaces parallel to the long 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. Degree of crystallinity values obtained using one set of standards and a particular experimental method are not likely to agree with values obtained using 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.

SUMMARY

1. Calcium gluceptate exists in both crystalline and non-crystalline forms and in each of these it can be an anhydrate or a hydrate containing 3 1/2 molecules of water of crystallization.

2. Up until the early part of 1980, calcium gluceptate was marketed as the amorphous anhydrate (III) but since this time the material available commercially has been the crystalline hydrate(I).

3. Dehydration of I resulted in a crystalline anhydrate (II). The apparent aqueous solubility of III was found to be > 2 molal while the apparent solubilities of I and II were 0.07 and 1.29 molal respectively.

4. Solutions prepared with II or III were unstable and precipitated on storage as I.

5. Stable calcium gluceptate solutions (≃25% w/v) were prepared from I by first dehydrating it to II and using II to prepare the solutions. The solutions were stabilized by autoclaving immediately after preparation.

6. Above 66% relative humidity (RH), I was the stable form and II was stable at 0% RH. The various solid phases can be interconverted by the processes of drying, grinding and storage at controlled RH.

7. I and II coexist at RH above 0% and below 67%. The adsorption of a small quantity of water vapor by II inhibits the transition to I by preventing further water uptake. This adsorption also prevents the melting of II.

8. Grinding II increased its apparent water solubility. Grinding decreased both particle size and the degree of crystallinity, but the latter was the major factor with respect to the increases in apparent water solubility.

9. The degree of crystallinity of II was assessed by x-ray, heat of solution and density measurements, but the values were not in good agreement.

10. 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 amorphous material in a crystalline-amorphous mixture (two-state model).

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APPENDIX I

Calculation of linear absorption coefficients of anhydrous calcium gluceptate and lithium fluoride

The linear absorption coefficient, μ_c, of a compound is given by the formula (Bragg, 1962):

$$\mu_c = \rho \Sigma p_e \cdot \mu_e/\rho_e \qquad (11)$$

where ρ is the density of the compound and p_e is the proportion by weight of each constituent element with mass absorption coefficient μ_e/ρ_e

(i) Anhydrous calcium gluceptate (II)

Molecular formula of II is $C_{14}H_{26}CaO_{16}$ (USP XX, 1980c)
Weight fractions of calcium, carbon, hydrogen and oxygen are 0.082,0.343,0.053 and 0.522 respectively.
The density of II is 1.66 g cm^{-3} (Table XI)
The mass absorption coefficients of calcium, carbon, hydrogen and oxygen for CuKα radiation are 171, 4.22, 0.391 and 11.0 cm^2 g^{-1} respectively (Cullity, 1978e).
The linear absorption coefficient of II

$= 1.66 \{(0.082 \times 171) + (0.343 \times 4.22) + (0.053 \times 0.391) + (0.522 \times 11.0)\} = 35.3$ cm^{-1}

(ii) Lithium fluoride

Weight fractions of fluorine and lithium in lithium fluoride (LiF) are 0.733 and 0.268 respectively.

Density of lithium fluoride is 2.64 g cm^{-3} (Merck Index, 1983a).

The mass absorption coefficients of fluorine and lithium for CuKα radiation are 16.0 and 0.477 cm^2 g^{-1} respectively (Cullity, 1978e).

The linear absorption coefficient of lithium fluoride

$= 2.64\{(0.733 \times 16.0) + (0.268 \times 0.477)\}$

$= 31.3\ cm^{-1}$

APPENDIX II

Calculation of hypothetical particle size of unground II and II ground for 1 h

The calculation of hypothetical particle size is based on the assumption that all the particles were spherical in shape and of uniform size.

(i) Unground II

Surface area = 5.45 $m^2 g^{-1}$ (Table XI)

= 5.45 x 10^4 $cm^2 g^{-1}$

Density =1.6621 g cm^{-3} (Table XI)

Volume of 1 g of II = 0.6017 cm^3

Let the number of particles in 1 g of powder be n_1. The surface area of 1 g of powder would be $4\pi r_1^2 n_1$ and its volume $4/3\pi r_1^3 n_1$, where r_1 is the radius of the particles.

$$\frac{\text{Area of 1 g of powder}(cm^2)}{\text{Volume of 1 g of powder}(cm^3)} = \frac{4\pi r_1^2 n_1}{4/3\pi r_1^3 n_1} = \frac{5.45 \times 10^4}{0.6017}$$

$$r_1 = 3.31 \times 10^{-5} \text{ cm}$$

diameter = 662 nm (6620 Å)

(ii) II ground for 1 h

Surface area = 10.51 $m^2 g^{-1}$(Table XI)

= 10.51 x 10^4 $cm^2 g^{-1}$

Density = 1.6822 g cm^{-3}(Table XI)

Volume of 1 g = 0.5945 cm^3

Let n_2 be the number of particles in 1 g of powder and r_2 be the radius of each particle.

$$\frac{\text{Area of 1 g of powder}(cm^2)}{\text{Volume of 1 g of powder}(cm^3)} = \frac{10.51 \times 10^4}{0.5945}$$

$$r_2 = 1.7 \times 10^{-5} \text{ cm}$$

diameter = 340 nm (3400 Å)

APPENDIX III

Calculation of the effect of particle size on the solubility of II

The Ostwald-Freundlich equation relates the solubility of a compound with its particle size and is given by (Florence and Attwood, 1982):

$$\log\frac{S_2}{S_1} = \frac{2\gamma M}{2.303RT\rho}\left(\frac{1}{r_2} - \frac{1}{r_1}\right) \qquad (12)$$

where S_1 is the solubility of particles of radius r_1 and S_2 is the solubility of particles of radius r_2 at a temperature T (°K); M, γ and ρ are the molecular weight, surface energy and density of the solid respectively.

The radius r_2 of 1 h ground particles was 1.7×10^{-5} cm and radius r_1 of unground particles was 3.3×10^{-5} cm (Table XI).

Molecular weight of II = 490.43

Density of II = 1.6621 g cm^{-3}

Temperature = 25°C = 298°K (assumed)

Since the surface energy of II is not known, arbitrary values ranging from 1×10^{-6} J cm^{-2} to 5×10^{-5} J cm^{-2} have been considered.

The calculated solubility ratio of the 1 h ground to unground sample would range from 1.01 : 1.00 where the surface energy is 1×10^{-6} J cm^{-2} to 1.41 : 1.00 where the surface energy is 5×10^{-5} J cm^{-2}.