Phase Transitions of Glycine in Frozen Aqueous Solutions and during Freeze-Drying

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Purpose. To study the solid-state and phase transitions of glycine, (i) in frozen aqueous solutions, and (ii) during freeze-drying.
Methods. X-ray powder diffractometry (XRD) and differential scanning calorimetry (DSC) were used to analyze the frozen systems. *In situ* freeze-drying in the sample chamber of the diffractometer enabled characterization of phase transitions *during* freeze-drying.
Results. *Transitions in frozen systems*. Rapid (20°C/min) or slow (2°C/min) cooling of aqueous solutions of glycine (15% w/w) to −70°C resulted in crystallization of β-glycine. Annealing at −10°C led to an increase in the amount of the crystalline phase. When quench-cooled by immersing in liquid nitrogen, glycine formed an amorphous freeze-concentrate. On heating, crystallization of an unidentified phase of glycine occurred at ~ −65°C which disappeared at ~ −55°C, and the peaks of β-glycine appeared. Annealing caused a transition of β- to the γ- form. The extent of this conversion was a function of the annealing temperature. Slower cooling rates and annealing in frozen solutions increased the crystalline β-glycine content in the lyophile. Freeze-drying of quench-cooled solutions led to the formation of γ-glycine during primary drying resulting in a lyophile consisting of a mixture of β- and γ-glycine. The primary drying temperature as well as the initial solute concentration significantly influenced the solid-state of freeze-dried glycine only in quench-cooled systems.
Conclusions. The cooling rate, annealing conditions and the primary drying temperature influenced the solid-state composition of freeze-dried glycine.

KEY WORDS: glycine; solid-state; frozen; freeze-drying; X-ray powder diffractometry; differential scanning calorimetry.

INTRODUCTION

To develop a rational freeze-drying cycle and to achieve product consistency, it is necessary to characterize the physical state of a solute, in frozen systems (1). If the solute is crystalline and forms a eutectic with ice, primary drying should be conducted below the eutectic melting temperature (T_e). If a solute remains amorphous, the frozen system is characterized by the glass transition temperature of the maximally freeze-concentrated solute, T_g'. Primary drying above T_g' can cause collapse of the freeze-dried cake. Annealing in frozen solutions is a process that facilitates crystallization. The annealing conditions influence not only the kinetics of crystallization, but also the polymorphic form of the crystallizing phase. Therefore the processing conditions (annealing temperature and time, and primary drying temperature) will be a function of the polymorphic form of the solute.

Glycine is a commonly used bulking agent in freeze-dried formulations. Three polymorphic forms of anhydrous glycine- α-, β- and γ- have been widely reported in the literature. Cooling of aqueous solutions usually results in the crystallization of β-glycine (1,2). This forms a eutectic mixture with ice, with a melting temperature of ~ −4°C. Freeze-dried systems usually contain β-glycine. However, at room temperature in the presence of moisture, β-glycine converts readily to mixtures of γ- and α-glycine. The low temperature thermal behavior of frozen glycine solutions were recently investigated (3). Using differential scanning calorimetry and freeze-drying microscopy, the effect of processing conditions (especially cooling rate and annealing temperature) on the solid-state of glycine in frozen solutions were elegantly illustrated. In spite of its widespread use, there is incomplete understanding of the phase behavior of glycine during freeze-drying. Moreover, there is no report in the pharmaceutical literature that deals with polymorphic transitions during the annealing of frozen solutions and during the primary drying steps. These are particularly important in light of the profound effect of formulation and processing variables on the physical state of the lyophile.

X-ray powder diffractometry (XRD) is a powerful technique for the routine characterization of pharmaceuticals (4). While differential scanning calorimetry has been traditionally used to study frozen aqueous solutions, low temperature XRD has proven to be an excellent tool to identify the phases that crystallize in the system (5). By attaching a vacuum pump to the low temperature stage of an X-ray diffractometer, it has been possible to carry out the entire freeze-drying process in the sample chamber of the XRD and monitor, in real time, the phase changes that occur during the different stages of the process.

Our first object was to determine the influence of processing conditions on the crystallization behavior of glycine *during* the various stages of the freeze-drying cycle. The processing conditions investigated were: (i) cooling rates, (ii) annealing conditions, and (iii) primary drying temperatures. The second object was to use XRD to quantitatively monitor the crystallization and polymorphic transitions as a function of the annealing time. The final objective was to perform similar quantitative analyses during primary drying.

EXPERIMENTAL

Materials

Glycine (Sigma Ultra, > 99% pure) was used as received.

Methods

X-Ray Powder Diffractometry

An X-ray powder diffractometer (Model XDS 2000, Scintag) with a variable temperature stage (Micristar, Model 828D, R.G. Hansen & Associates; working temperature range −190°C to 300°C) was used. The controlled temperature stage of the XRD was attached to a vacuum pump. As a result it was possible to carry out the entire freeze-drying process in

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the sample chamber of the XRD. An accurately weighed amount of solution (100 mg) was filled into a copper sample holder and cooled at a constant predetermined rate (2 or 20°C/min) from room temperature to −70°C. The solution concentration was 15% w/w unless otherwise specified.

When quench-cooling was desired, the solution was filled in the copper holder and lowered in a shallow bath of liquid nitrogen. After the solution had completely frozen, the holder containing the sample was removed and placed on the temperature stage which was preset at −90°C. The frozen samples were then subjected to a controlled temperature program. The specific details are provided in the Results and Discussion Section. XRD patterns were obtained by exposing the sample to CuKα radiation (45 kV × 40 mA), wherein the scanning speed was 5°2Θ min^{-1} and the step size was 0.03°2Θ. During the XRD runs, unless otherwise mentioned, the samples were maintained under isothermal conditions at the selected temperatures. The frozen solution was then subjected to primary drying *in situ* in the sample chamber of the XRD at a pressure of ~ 100 mTorr. Again, the details are provided in the Results and Discussion section. Primary drying was carried out until all the ice was removed. The sample was then heated to the secondary drying temperature of 25°C where the drying was continued for the desired time period.

Differential Scanning Calorimetry (DSC)

A DSC equipped with a liquid nitrogen cooling system (Model Pyris 1, Perkin Elmer) was used. Helium was used as the purge gas and the flow rate was 40 ml/min. The DSC was calibrated using indium, mercury and distilled water as standards. About 10–15 mg of each solution was weighed in an aluminum pan which was hermetically sealed. The solutions were cooled at a controlled rate (2 or 20°C/min) to the desired temperature, held for 20 min and then heated to 25°C at 5°C/min. For investigating quench-cooled solutions, the aqueous solutions of glycine were cooled at a programmed rate of 500°C/min to −90°C. The actual cooling rates were slower. However, the DSC heating profile of this sample was virtually identical to that of the quench-cooled sample.

RESULTS AND DISCUSSION

Based on X-ray powder diffractometry (XRD), differential scanning calorimetry (DSC) and thermogravimetric analysis, the 'as is' glycine was identified as the anhydrous α-polymorph.

Influence of Controlled Cooling

When 15% w/w aqueous glycine solution was cooled (either at 20 or at 2°C/min) to −70°C in the sample stage of the XRD, peaks due to crystalline hexagonal ice (at 22.5°, 24.1 and 25.5°2Θ) and β-glycine (at 18.1, 23.6 and 28.6°2Θ) were observed (Fig. 1; top panel; XRD pattern at −70°C). When the frozen solution was heated at 5°C/min, the eutectic melting at ~ −4°C was followed immediately by ice melting (not shown). At the eutectic melting temperature, the peaks of β-glycine disappeared and there was a pronounced decrease in the intensities of ice peaks. Thus low temperature XRD provided direct evidence of the appearance as well as the disappearance of the crystalline phases. These results were confirmed by DSC.

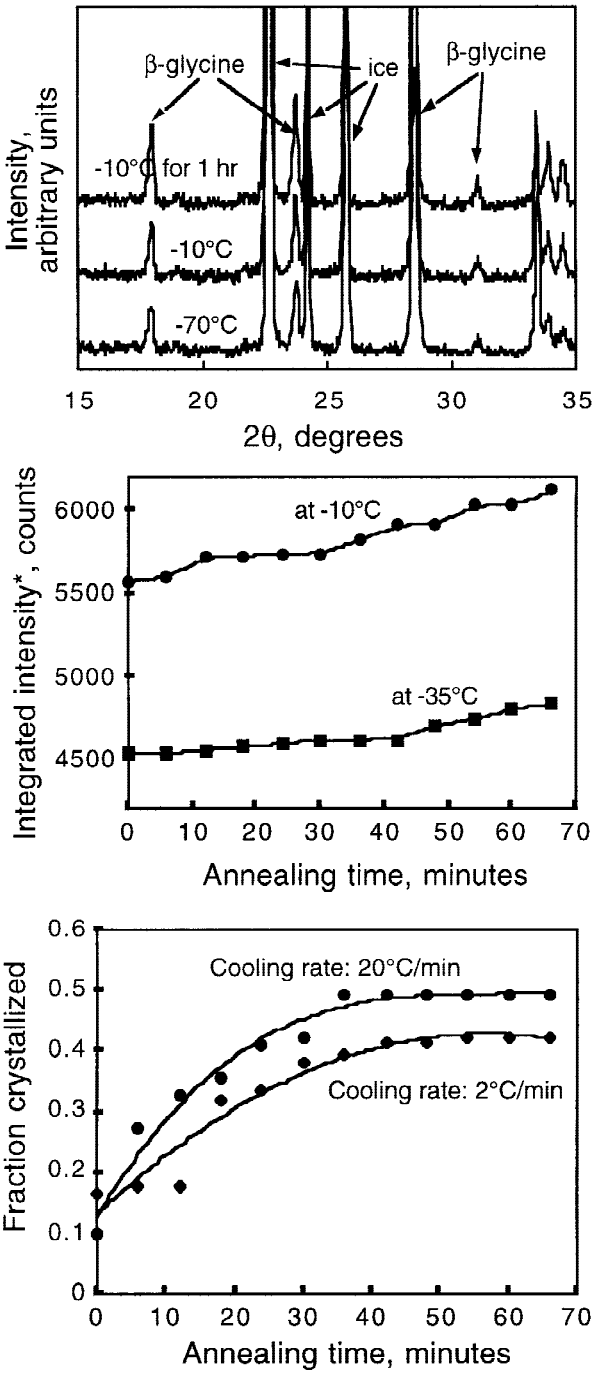

Fig. 1. Kinetics of crystallization of glycine as a function of annealing time. In case of the top and middle panels, 15% w/w glycine solutions were cooled at 20°C/min to −70°C and then heated at 5°C/min to the annealing temperature. Top panel: Overlaid XRD patterns of frozen aqueous solutions of glycine. Peaks unique to β-glycine and hexagonal ice have been pointed out. Middle panel: Effect of annealing temperature. Annealing was carried out either at −35°C or at −10°C; *sum of the integrated intensity of the peaks at 18.1°2Θ and 28.6°2Θ. Bottom panel: Effect of cooling rate. The solution was cooled either at 2°C/min or at 20°C/min and annealed at −35°C. The curves are drawn to assist in visualizing the trends.

Our first interest was to determine the effect of annealing temperature on the kinetics of glycine crystallization in frozen aqueous solutions. XRD was ideally suited for this purpose. As before, an aqueous solution of glycine was cooled from

room temperature to −70°C either at 2 or at 20°C/min. It was then warmed to the annealing temperature at 5°C/min. Annealing was carried out either at −35 or at −10°C. For example, after annealing at −10°C for 1 h, there was a pronounced increase in the intensities of β-glycine peaks (Fig. 1; top panel). The sum of the integrated intensities of the 18.1° and 28.6°2Θ peaks of β-glycine were used as a measure of the amount of β-glycine that had crystallized during annealing (Fig. 1; middle panel). It is evident that more β-glycine existed in the crystalline state at the higher annealing temperature.

The effect of cooling rate on the crystallization kinetics was also determined by plotting the fraction crystallized as a function of the annealing time (Fig 1; bottom panel). Annealing was carried out at −35°C. The fraction crystallized at any time 't' was expressed as: (sum of the integrated intensities of the 18.1 and 28.6°2Θ peaks of glycine at time 't')/ (sum of the intensities of the same peaks after annealing for 2 hours). Based on several experimental runs, we concluded that the cooling rate did not influence the crystallization kinetics. The bottom panel in Fig. 1 is one representative example. Similar results (data not shown) were obtained when solutions were annealed at −10°C. However, as expected, at all time points, annealing at −10°C caused a larger fraction of glycine to crystallize than at −35°C. Thus at both the cooling rates, glycine crystallized but incompletely during cooling. Both during cooling and annealing, only β-glycine crystallized from solution.

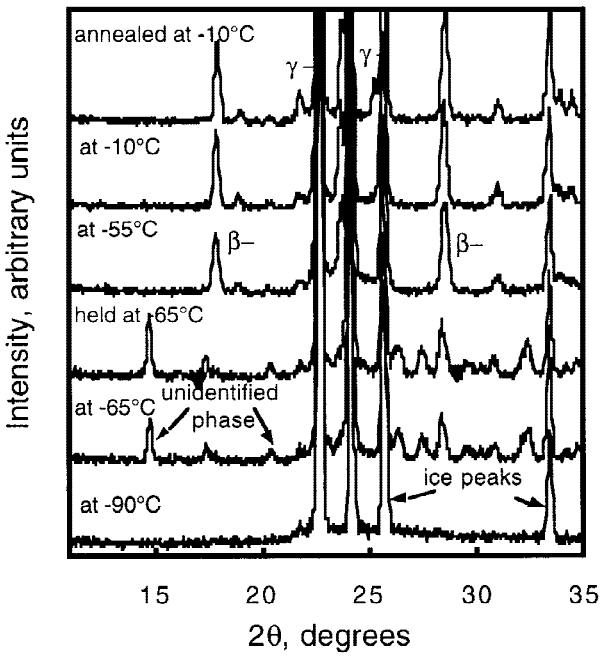

Fig. 2. XRD patterns of quench-cooled frozen aqueous solution of glycine (15% w/w) at different temperatures. Glycine solution was quench-cooled in liquid nitrogen. It was then heated at 5°C/min and XRD patterns were obtained from −90° to −10°C. Peaks unique to each of the glycine phases have been pointed out.

Influence of Quench-Cooling

The aqueous solutions of 15% w/w glycine were cooled by immersing in liquid nitrogen and placed on the sample stage at −90°C. The frozen sample was heated and the XRD patterns were obtained at different temperatures (Fig. 2). When the samples were subjected to variable temperature XRD, the temperature was maintained constant *during* the XRD run. Typically, an XRD run over the angular range of 10 to 35°2Θ was complete in 5 min. In this case, the samples were also subjected to nonisothermal XRD, wherein XRD patterns were continuously obtained while the sample was heated at 0.5°C/min. Qualitatively, very similar results were obtained in the two cases.

The pattern at −90°C revealed only peaks of ice, indicating that the solute had not crystallized and had formed an amorphous freeze-concentrate. At −65°C, some new peaks (for example at 14.5, 17.5, and 20.0°2Θ) appeared, strongly suggesting crystallization of glycine from the amorphous freeze-concentrate. However, their d-spacing values did not match with those of any of the known phases of glycine. We therefore postulate that this is an unidentified glycine phase. On heating further, at ~ −55°C, these peaks of glycine disappeared and those of β-glycine appeared. This observation strengthens our postulate that the unidentified phase is a polymorphic form of glycine. It is probably quite unstable and readily transforms to β-glycine. No other transitions were observed until the eutectic melting. The DSC profile of the quench-cooled solution showed a complex glass transition with the midpoint at ~ −75°C (Fig. 3). This was followed by two exotherms at ~ −55 and at −45°C. The first exothermic transition may be attributed to crystallization of the amorphous freeze-concentrate. The temperature of crystallization in the DSC (~ −55°C) was higher than that in the XRD (~ −65°C). This disagreement may be due to differences both in the sample size and the temperature program in the two instruments. The second exothermic transition in the DSC is attributed to the polymorphic transition of the unidentified phase to β-glycine.

Annealing

Glycine solution (15% w/w), placed in an XRD holder, was quench-cooled in liquid nitrogen. The holder was then transferred to the temperature stage which was set at −90°C. It was heated to −10°C at 5°C/min. The presence of β-glycine was evident from the peaks at 18.1, 23.6, and 28.6° 2Θ (data not shown). The XRD patterns were then obtained as a func-

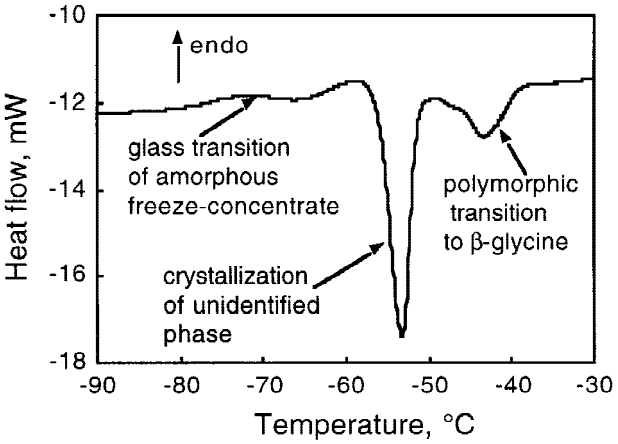

Fig. 3. DSC heating profile of frozen aqueous solution of glycine (15% w/w). The solution was cooled from room temperature to −90°C at 500°C/min.

tion of annealing time. There was a progressive decrease in the intensities of the β-glycine peaks and this was accompanied by an increase in the intensities of the γ-glycine peaks, for example at 21.8 and 29.4°2Θ. Thus the formation of γ-glycine occurred at the expense of β-glycine.

DSC confirmed the results obtained by XRD. The DSC curve of a solution that was slowly cooled and annealed consisted of two endotherms: the first (~ −4°C) is attributed to the eutectic melting of glycine (Fig. 4; upper panel). The second endotherm at ~ 0°C is likely an experimental artifact attributed to melting of ice that condensed on the lid inside the DSC pan (7). In case of the quench-cooled solution, an additional endotherm (at ~ −3°C) due to the eutectic melting of γ-glycine was observed (Fig. 4; lower panel).

Our next objective was to quantify the glycine phases crystallizing during annealing and thereby approximate the kinetics of crystallization (Fig. 5). This experiment was carried out at several annealing temperatures. When annealed at −10°C, there was a progressive decrease in the intensity of glycine peak for ~150 min and a concomitant increase in the intensity of γ-glycine peak. Annealing at −20°C resulted in a gradual increase in the fraction of β-glycine. Whereas crystalline γ-glycine was detected at this temperature, its fraction was very low and showed a slight increase over a time period of ~ 250 min. At −35°C, a similar observation was made with regard to β-glycine. At this temperature, γ-glycine was barely detected. It was also of interest to anneal the frozen solution containing the unidentified phase which was discussed earlier (Fig. 2; phase observed at −65°C). When the annealing was conducted at −65°C for 2 hours, while a small increase in the peak intensities was observed, there was no evidence of any phase transitions (data not shown).

The complex thermal behavior of frozen glycine solutions was elucidated by Chongpresart *et al.* (3). When the solute was cooled at a controlled rate of 2°C/min and annealed at −3.9°C, there was formation of α-glycine. However, when the solution was quench-cooled and annealed at −2.9°C, γ-glycine was obtained. By ingenious manipulation of the thermal history, the complicated thermal events in the eutectic region were elucidated. We carried out annealing at temperatures lower than the eutectic temperature of β-glycine. We observed polymorphic transition of β-glycine→ γ-glycine during annealing of only quench-cooled solutions. Irrespective of the annealing temperature, the thermal history seems to significantly influence the phase behavior of frozen systems.

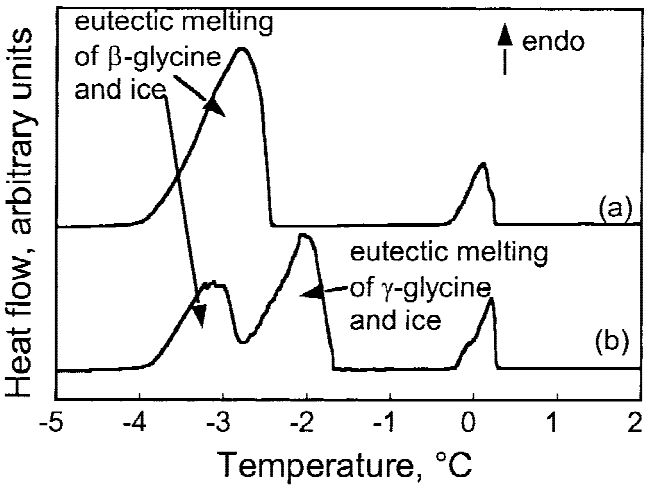

Fig. 4. DSC curves of frozen aqueous solutions of glycine (15% w/w) after annealing at −10°C for ~ 2 h. (a) Solution cooled to −70°C at 2°C/min and heated to −10°C at 5°C/min and then to 5°C at 0.5°C/min. (b) Solution cooled to −90°C at 500°C/min and heated to −10°C at 5°C/min and then to 5°C at 0.5°C/min.

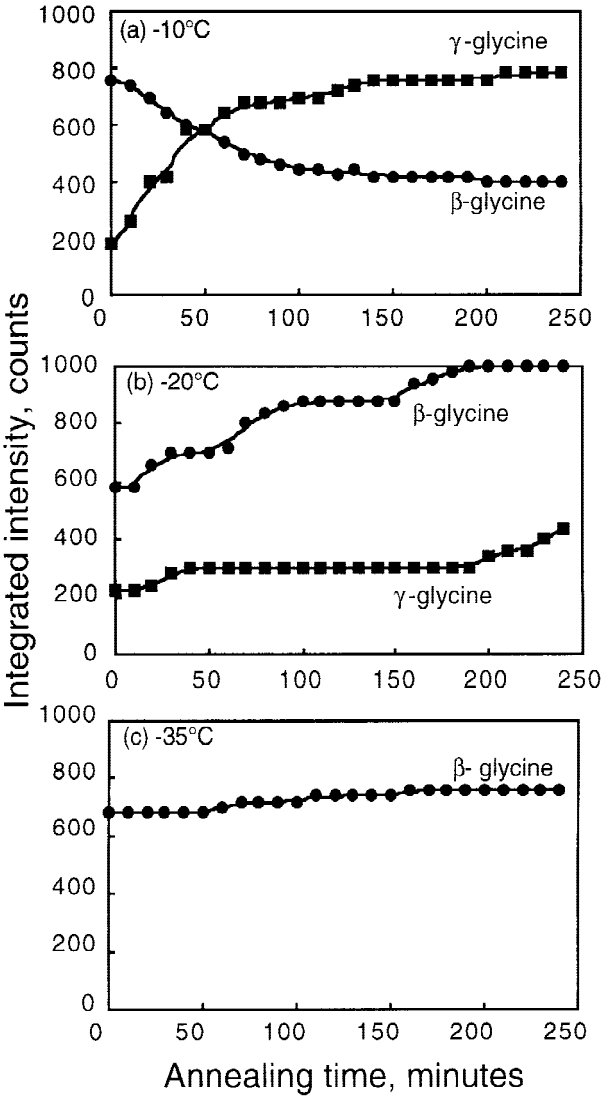

Fig. 5. Plots of the integrated intensity of the peaks of β-glycine (peak at 18.1°2Θ) and γ-glycine (peak at 21.8°2Θ) as a function of annealing time. The annealing temperature were (a) −10°C , (b) −20°C and (c) −35°C. 15% w/w aqueous solutions were quench-cooled by immersing in liquid nitrogen and heated to the annealing temperature at 5°C/min.

Among the three polymorphic forms of glycine, α-, β-, and γ, β-glycine is reported to be the most unstable at room temperature, especially under moist conditions (1,3). The stability relationships between the different polymorphs at subambient conditions is not known. However, based on the observed solubilities of α- and γ- glycine over the temperature range of 10 to 70°C (8), two conclusions can be drawn: (i) γ-glycine is the stable form in this temperature range (3) and (ii) γ-glycine will continue to be the stable form for at least 50°C on each side of the temperature range studied. There-

fore in the temperature range of annealing (–10 to –35°C), γ-glycine is likely to be the stable form. This is supported by the observation that very slow cooling (0.1°C/min) of aqueous solutions of glycine led to the formation of γ-glycine (3).

When the solutions were quench-cooled and heated, the following stepwise transitions: amorphous ⇨ unidentified glycine phase → γ → γ were observed. This sequence of transitions fits well with Ostwald's step rule, "In all processes, it is not the most stable state with the lowest amount of free energy that is initially formed, but the least stable state lying nearest in free energy to the original state" (9). While quench-cooling followed by heating resulted in a mixture of the stable (γ-) and metastable (β-) polymorphs (Fig. 2), slower cooling followed by heating resulted only in the metastable β-form (Fig. 1). We hypothesize that, in quench cooled systems, when the unidentified glycine phase converts to β-glycine, simultaneous nucleation of γ-glycine also occurs. On annealing the system at –10°C, the nuclei of γ- grow and as the relative stability of β- is lower than that of γ- at that temperature, there is a β → γ transition.

Transitions during Freeze-Drying

In situ XRD enabled us to monitor in real-time the phase transitions that occurred during the process of freeze-drying.

Effect of Cooling Rate

Our first interest was to determine the influence of cooling rate on the physical state of the lyophile. Glycine solution was cooled either at 2 or 20°C/min to –70°C. It was warmed to –10°C at 5°C/min and then subjected to primary-drying. Glycine solution was also quench-cooled to –90°C using liquid nitrogen. This was also warmed to –10°C at 5°C/min and freeze-dried. While controlled cooling led to the formation of β-glycine in the lyophile, quench-cooling resulted in a mixture of γ- and β-glycine (Fig. 6).

The integrated intensities of the glycine peaks were monitored as a function of primary drying time (Fig. 7). The amount of γ-glycine increased rapidly in the first 10 min while the amount of β-glycine increased gradually and continuously over the first hour. Therefore, during primary-drying, both β- and γ- glycine crystallized from the freeze-concentrate. This behavior was in contrast to that observed during annealing of quench-cooled solutions of glycine wherein the concentration of γ-glycine increased at the expense of β-glycine (Fig. 5).

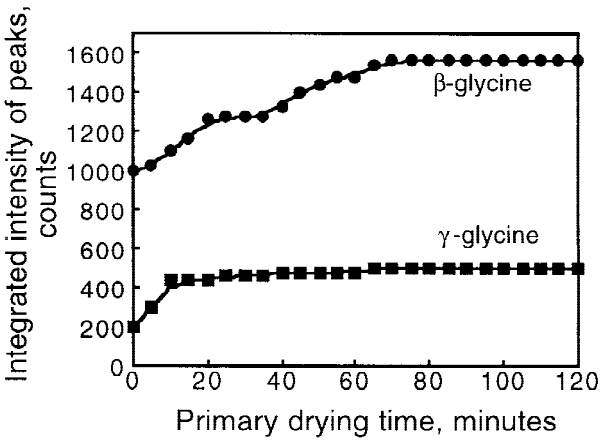

Fig. 7. Plots of the integrated intensity of the peaks of β-glycine (peak at 18.1°2Θ) and γ-glycine (peak at 21.8°2Θ) during primary drying at –10°C. Aqueous solution of glycine (15% w/w) was quench-cooled and then warmed from –90°C to –10°C at 5°C/min.

Effect of Primary Drying Temperature

When glycine solution was cooled at a controlled rate, the primary drying temperature did not affect the solid-state of the lyophile. When primary drying was carried out at –35, –20 or at –10°C, β-glycine of nearly identical solid-states were obtained. In the case of quench-cooled solutions, the primary drying temperatures affected the solid-state of the lyophile. While the β-glycine content in the lyophiles was approximately constant and independent of the primary drying temperature, an increase in the primary drying temperature resulted in an increase in the γ-glycine content (Fig. 8). Thus, an increase in the primary drying temperature increased the crystalline content in the lyophile.

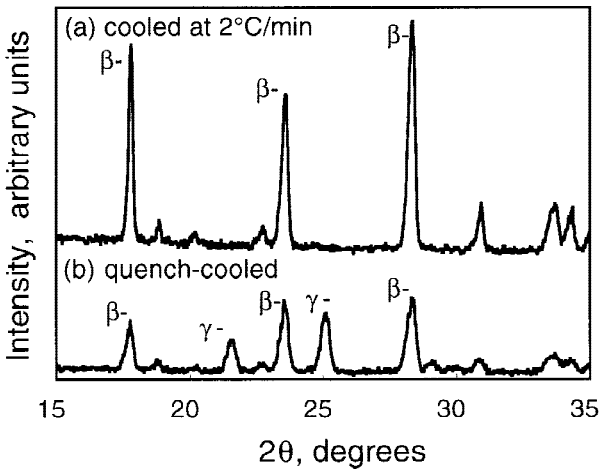

Fig. 6. Effect of cooling rate on solid-state of freeze-dried glycine. (a) Cooling at 2°C/min led to formation of lyophile that contained β-glycine. (b) Quench-cooling resulted in a mixture of β- and γ-glycine. In both cases, the glycine concentration was 15% w/w and the primary drying temperature was –10°C.

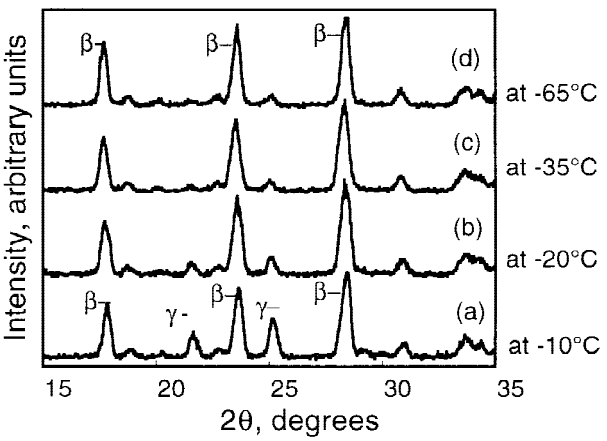

Fig. 8. Overlaid XRD patterns of lyophiles obtained after quench-cooling (15% w/w aqueous solution of glycine) and primary drying at different temperatures. The primary drying temperatures were: (a) –10°C, (b) –20°C, (c) –35°C, and (d) –65°C.

Effect of Initial Solute Concentration

When the solutions were cooled at a controlled rate (either at 2 or at 20°C/min), irrespective of the initial solute concentration (5 to 15% w/w), the lyophile consisted only of β-glycine. As mentioned earlier (Fig. 8), lyophiles obtained after quench-cooling 15% w/w glycine solutions, contained β- and γ-glycine. The proportions of the two depended on the primary drying temperature (Fig. 8). At low glycine concentration (5% w/w) and at low primary drying temperatures (−65 or −45°C), only β-glycine was detected. However, primary drying at −35°C or at higher temperatures yielded mixtures of β- and γ- glycine. These results indicated that the solid-state of the lyophile was a complex function of (i) the cooling rate, (ii) the primary drying temperature and (iii) the initial solute concentration.

Practical Significance

While the physical state of the solute is influenced by both the processing conditions and the formulation variables, the present investigation has focussed only on the former. This includes the study of polymorphism, partial or complete crystallization of an amorphous phase, and phase transformations during freeze-drying. A knowledge of the phase transitions of the solute during the freeze-drying cycle will enable the identification of critical process variables and lead to a consistent product that meets regulatory requirements.

As pointed out earlier, γ-glycine appears to be the stable form at ambient temperature (3,8). The freeze-dried glycine, irrespective of the processing conditions, contained β-glycine—a phase believed to be metastable at room temperature. However mixtures of β-glycine and γ-glycine were obtained following the freeze-drying of quench-cooled solutions. There are two interesting observations here. (i) The glycine phase most frequently encountered (β-glycine) is metastable. (ii) This metastable phase also readily exists in presence of the stable phase (γ-glycine). The presence of a metastable phase raises some important questions. Since metastable phases tend to be more reactive than their stable counterparts, can this have any adverse impact on the dosage form? Secondly, does the metastable phase undergo any phase transition during storage of the dosage form? To the best of our knowledge, these issues have not been addressed in the literature.

Glycine is believed to crystallize readily during freeze-drying. However, when cooled at a relatively slow rate of 2°C/min, glycine did not crystallize completely during cooling. This is evident from the increase in the amount of crystalline glycine due to annealing (Fig. 1, bottom panel). The presence of other excipients including sucrose may also severely inhibit glycine crystallization (10). Incomplete solute crystallization can result in a dramatic decrease in T_g' (the glass transition temperature of the maximally freeze-concentrated solute), and reduce the collapse temperature (which is usually very close to T_g') to low values making freeze-drying impractical (11). These characterization studies will not only facilitate the identification of the annealing temperature but will also reveal the annealing time required for maximum crystallization (for example, Fig. 1 bottom panel). Though the present investigation deals with glycine, a widely used pharmaceutical excipient, similar studies may be warranted for other drugs and excipients. In fact, similar studies with mannitol, revealed the existence of mannitol hydrate during freeze-drying (12).

Assessment of the Available Techniques

DSC has been extensively used to characterize frozen systems. The technique is very sensitive and provides information about both crystalline and amorphous phases. The drawbacks of the technique are: (i) inability to directly identify crystalline phases and (ii) difficulty in interpretation when there are complex or overlapping thermal events. While XRD lacks the sensitivity of DSC and is unsuitable for the characterization of amorphous phases, it enables simultaneous identification of multiple crystalline phases. In addition, it provides information about the crystallinity of the phases (5,12). Freeze-drying microscopy enables direct observation of the freezing and the freeze-drying processes and can therefore be used to determine the collapse temperature (13).

Only DSC and XRD can provide quantitative information. DSC has been used to study the extent of crystallization of the amorphous freeze-concentrate during annealing (14,15). The frozen sample is heated to the annealing temperature, T_a (usually above T_g'), and maintained for the desired time period. The sample is then cooled to a temperature below T_g' and reheated. During the reheating, the remaining amorphous solute will crystallize, at a temperature $T >> T_g'$ (note that $T > T_a$). The enthalpy value is a measure of the fraction of solute that did not crystallize during annealing (14). In a completely different approach, the enthalpy of eutectic melting was used to quantify the solute crystallization (15).

XRD is ideally suited to monitor the extent of crystallization under isothermal conditions. Moreover, in situations where crystallization occurs in different stages, XRD can detect the events and provide quantitative information. It is also possible to monitor phase transitions *in situ* during the entire freeze-drying process by XRD. The major limitation of XRD is that it can only be used for the identification and quantification of crystalline phases, and there are numerous sources of error in quantitative XRD (4).

CONCLUSIONS

The effect of thermal history on the phase behavior of glycine during freeze-drying has been investigated. Even at a slow cooling rate of 2°C/min, glycine did not crystallize completely during cooling. Annealing facilitated glycine crystallization. In addition, in quench-cooled solutions, transition of β-glycine to γ-glycine occurred during annealing at −10°C. This transition was not observed at lower annealing temperatures. When glycine solutions were cooled at a controlled rate and freeze-dried, the lyophile consisted of β-glycine. On the other hand, when it was quench-cooled, the lyophile consisted of β- and γ-glycine. Finally, the primary drying temperature also influenced the solid phase composition. Thus the solid-state of the lyophilized glycine appeared to be a complex function of the cooling rate, annealing conditions and the primary drying temperature. The *in situ* XRD technique, coupled with DSC, permitted phase characterization during all the stages of the freeze-drying process.

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