The Effect of Additives on the Crystallization of Cefazolin Sodium during Freeze-Drying

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Purpose. To monitor the phase transitions during freeze-drying of cefazolin sodium (I) as a function of process and formulation variables.

Methods. Aqueous solutions of I were frozen under controlled conditions in the sample chamber of a variable temperature X-ray powder diffractometer (XRD). The instrument was modified so that the chamber could be evacuated and the samples dried under reduced pressures. Thus, the entire freeze-drying process was carried out in the XRD holder with real time monitoring of the phase transitions during the different stages of freeze-drying.

Results. When aqueous solutions of cefazolin sodium (10% w/w) were cooled to –40°C, the XRD pattern revealed only the crystallization of ice. Annealing the frozen sample led to the crystallization of I as the pentahydrate. Differential scanning calorimetry revealed that the presence of isopropyl alcohol (IPA) (5% w/w) led to a decrease in the Tg′, the glass transition temperature of the system, and lowered the temperature of crystallization. The crystallization was studied at –8 and at –15°C in the XRD, and, as expected, more rapid crystallization was observed at the higher temperature. Primary drying at –8°C led to the dehydration of the pentahydrate, resulting in a poorly crystalline product. Again, XRD permitted real time monitoring of the decrease in intensities of some characteristic peaks of the pentahydrate. The *in situ* XRD technique also enabled us to study the effects of processing conditions (different primary and secondary drying temperatures) and crystalline bulking agents on the solid-state of I in the lyophile. When I was lyophilized using mannitol or glycine as an additive, without an annealing step, the drug was X-ray amorphous although the additive crystallized. When annealed and freeze-dried, I remained crystalline in the presence of glycine but not in the presence of mannitol.

Conclusions. The *in situ* XRD technique has enabled us to characterize the phase transitions during freeze-drying of cefazolin sodium in multicomponent systems.

KEY WORDS: cefazolin sodium; mannitol; glycine; crystallization; X-ray powder diffractometry; differential scanning calorimetry.

INTRODUCTION

When formulated as aqueous solutions, the shelf life of many antibiotics, including β-lactams, is unacceptably short (1). Most of these molecules undergo hydrolysis in aqueous solutions. Under these circumstances, there are two possible approaches to prepare products with adequate shelf life. (i) Formulation as a premixed frozen solution. These solutions are prepared, immediately frozen and stored at ≤ –20°C. The product is thawed to room temperature right before use. (ii) Formulation as a freeze-dried sterile powder which is reconstituted before administration (1,2).

In frozen solutions, the drug may not undergo crystallization. It then forms a freeze-concentrate and remains associated with an excess amount of unfrozen water. Decomposition reactions such as hydrolysis may be enhanced in the freeze-concentrated solute. Pikal *et al.* have shown that these decomposition reactions occur more readily in the amorphous state than in their crystalline counterparts (3). Thus, the solid state of the active ingredient in the formulation influences its stability in the dosage form. When sodium ampicillin solutions were stored at –20°C, significant drug decomposition occurred in as little as 48 h (4). This instability was attributed to the existence of the drug in a freeze-concentrated state since it had not crystallized from solution.

When compared with premixed frozen solutions, freeze-dried dosage forms offer several advantages. First, complete removal of water at low temperatures is possible. This reduces the possibility of decomposition due to the presence of water during storage. It is important to point out here that the stability may be considerably enhanced if the drug can be obtained in the crystalline state after freeze-drying. Secondly, freeze-dried dosage forms need not be stored at low temperatures. Moreover, this process is compatible with sterile procedures and therefore may be the method of choice for the preparation of parenterals (5).

It is now well known that the physical state of a solute in frozen and freeze-dried systems depends on the processing conditions and formulation variables. Several reports in the literature address the freeze-drying behavior of antibiotics (6–9). It is usually desirable to crystallize the solute in the frozen state, that is, before the beginning of primary drying. For example, it has been demonstrated that sodium nafcillin undergoes crystallization during annealing (7). In annealing, a process that facilitates crystallization, frozen aqueous solutions are held above the Tg′ but below the eutectic melting temperature. If nafcillin sodium remained in the amorphous state before freeze-drying, it resulted in a liquid crystalline phase that was less stable than the crystalline material.

Many antibiotics, which are candidates for lyophilization, do not crystallize easily in frozen and freeze-dried systems (2,4). Since these solutes are usually very soluble in water, they tend to remain in an unsaturated state in frozen aqueous solutions, and this prevents crystallization. Approaches such as annealing of frozen solutions aid in crystallizing more ice, resulting in a decrease in unfrozen water, enhancing supersaturation, and facilitating nucleation (10). The addition of an organic cosolvent or a crystallizing cosolute, especially amino acids, has been known to induce crystallization of the active ingredient in the final product (6,11,12). The choice of formulation variables also dictates the solid-state, freeze-drying behavior, stability, and elegance of the freeze-dried product.

To develop rational freeze-drying cycles, it is important to understand the implications of the processing conditions on the formulation. Previous work in our laboratory has demonstrated the utility of low temperature X-ray diffractometry in the characterization of frozen solutions (13). By attaching a vacuum pump to the low temperature stage of the X-ray diffractometer, the entire process of freeze-drying could be car-

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ried out in the sample chamber of the XRD (7). This enabled us to monitor in real time the phase transitions that occurred during the entire freeze-drying process. Freeze-dried pharmaceutical formulations are often multicomponent systems. Characterization of these systems is difficult when they contain both crystallizing and noncrystallizing solutes. The presence of a large proportion of water further complicates the issues. Low temperature XRD and differential scanning calorimetry (DSC), being excellent complementary techniques, provide an approach that may aid in obtaining an in-depth understanding of these complex systems.

Our main interest in this study was to investigate the effects of formulation and processing conditions on the solid state of a cephalosporinic antibiotic, cefazolin sodium. This drug can exist in the amorphous state, as a monohydrate ($C_{14}H_{13}N_8O_4S_3Na \cdot H_2O$), as a pentahydrate ($C_{14}H_{13}N_8O_4S_3Na \cdot 5H_2O$), and in "dehydrated forms" of both the monohydrate and the pentahydrate (6,14,15). These dehydrated forms have lower crystallinity compared with the corresponding crystalline hydrates. On freeze-drying, cefazolin sodium forms a poorly crystalline product. The presence of isopropyl alcohol and thermal treatment in the frozen state has been known to enhance the crystallinity of the lyophile (6). The addition of amino acids such as glycine enhanced the crystallinity of the freeze-dried cefazolin sodium (12). However, in all these studies, only the finished product was characterized. In other words, transitions during the various stages of the freeze-drying were not studied. Therefore, the mechanism by which the additives affect the crystallization of cefazolin sodium is unknown. Moreover, since the crystallinity of the lyophile was determined by polarized light microscopy, only qualitative information was obtained.

The broad goal of the project was to use cefazolin sodium as a model compound to investigate the effects of organic cosolvents and crystallizing cosolutes in the solid state of the active ingredients during the various stages of the freeze-drying process. We also were specifically interested in developing approaches to retain cefazolin sodium in a crystalline state at the end of the freeze-drying cycle.

MATERIALS AND METHODS

Materials

Cephazolin sodium ($C_{14}H_{13}N_8O_4S_3Na$), D-mannitol (Sigma Ultra), and glycine (Sigma Ultra) were obtained from the Sigma Chemical Co. and were used as received. Isopropyl alcohol (AR grade) was obtained from the Fischer Scientific Co.

Methods

X-Ray Powder Diffractometry

An X-ray powder diffractometer (Model XDS 2000, Scintag, Cupertino, CA) with a variable temperature stage (Micristar, Model 828D, R.G. Hansen & Associates, Santa Barbara, CA; working temperature –190°C to 300°C) was used. By attaching a vacuum pump to the temperature stage of the XRD (pressure ~100 mTorr), the entire freeze-drying process could be carried out in the sample chamber of the XRD. One hundred mg of aqueous cefazolin sodium solution (10% w/w) was accurately weighed into a copper sample holder and cooled from room temperature to –40°C at 10°C/min. It was held for 30 min and heated to the annealing or primary drying temperature at 5°C/min. The experimental conditions were different when IPA was used as a cosolvent and also when the crystallizing bulking agents (mannitol or glycine) were present. The specific details are provided in the Results and Discussion section. XRD patterns were obtained by exposing the sample to CuKα radiation (45 kV x 40 mA), wherein the scanning speed was $5°2\theta\ min^{-1}$ 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.

Drying

The frozen solution was then subjected to primary drying in the sample chamber of the XRD. The pressure was ~100 mTorr. Unless otherwise mentioned, secondary drying of the system was not carried out.

Differential Scanning Calorimetry (DSC)

A DSC equipped with a liquid nitrogen cooling system (Pyris 1, Perkin Elmer, Shelton, CT) was used. About 10–15 mg of the solution was weighed in an aluminum pan and was nonhermetically crimped. Helium, at a flow rate of 40 ml/min, was used as a purge gas. The DSC was calibrated using indium and distilled water as standards. Aqueous solution of cefazolin sodium (10% w/w) was cooled from room temperature to –50°C at 10°C/min. It was held for 30 min and heated to the annealing or primary drying temperature at 5°C/min. The experimental conditions were different when IPA was used as a cosolvent and also when the crystallizing bulking agent (mannitol or glycine) was present. The specific experimental details are provided in the Results and Discussion section.

RESULTS AND DISCUSSION

Characterization of Cephazolin Sodium

The "as is" cefazolin sodium characterized by XRD, DSC, and thermogravimetric analysis (TGA), was a partially crystalline monohydrate. The water content was determined to be ~3.5% w/w. On exposing it to 80% RH at room temperature, complete conversion to the pentahydrate was observed (water content of ~15% w/w). The XRD patterns of both the monohydrate and the pentahydrate were in excellent agreement with those reported in the literature (6).

Effect of Isopropyl Alcohol

Transitions in Frozen Aqueous Solutions

The first step in the freeze-drying process is the cooling of the solution. It was of interest to determine if the solute crystallized when a solution of cefazolin sodium was cooled. Therefore, an aqueous solution (10% w/w) of cefazolin sodium was cooled from room temperature to –40°C at 10°C/min. The XRD pattern revealed peaks at 22.5, 24.0 and 25.6° 2θ, all attributable to hexagonal ice. There was no crystallization of solute (Fig. 1, lowermost XRD pattern). As mentioned earlier, it has been reported that thermal treatment induced crystallization of cefazolin sodium in the frozen state. This process is also referred to as annealing and is usually carried out above the Tg′ of the system. DSC results

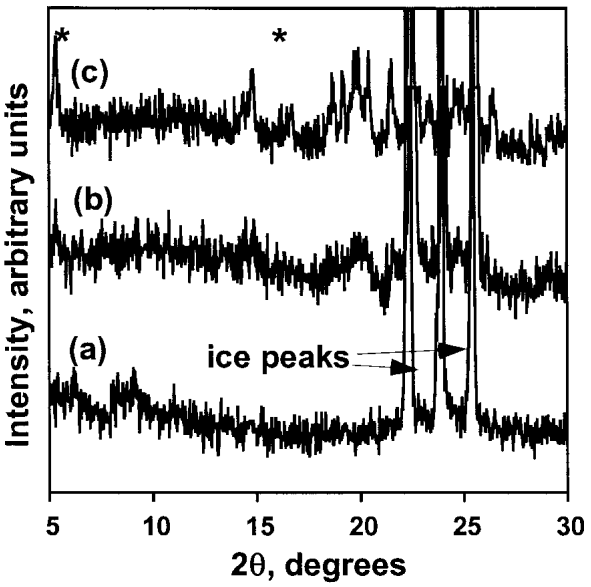

Fig. 1. XRD patterns of frozen aqueous solutions of cefazolin sodium (10% w/w) (a). The solution was cooled from room temperature to –40°C at 10°C/min (b). It was then heated to –8°C at 5°C/min and annealed for 1.5 h (c). Solution containing cefazolin sodium (10% w/w) and isopropyl alcohol (5% w/w) was cooled to –40°C at 10°C/min and then heated to –8°C at 5°C/min and annealed for 1.5 h. In (b) and (c), the XRD patterns were obtained at the end of annealing. Some characteristic peaks of cefazolin sodium pentahydrate are marked with an "*".

showed that the Tg′ of frozen aqueous solutions of cefazolin sodium was ~ –22°C (Fig. 2, upper panel), close to the reported value of –20°C (16). The frozen aqueous solution was heated from –40°C to –8°C in the XRD and annealed for 2 h. This led to solute crystallization, and the XRD pattern matched that of cefazolin sodium pentahydrate (Fig. 1b). However, the crystallization was substantially incomplete.

Next, we were interested in investigating the effect of isopropyl alcohol on the solid-state behavior of cefazolin sodium in frozen aqueous solutions. Thermal treatment in the presence of IPA induced the crystallization of cefazolin sodium (6). The phase diagram of an isopropyl alcohol-water system shows that IPA does not crystallize when cooled to -40°C (17). Thus, it is likely to form part of the amorphous freeze-concentrate. It also was evident from the phase diagram that on cooling aqueous solutions of 5% w/w IPA to –40°C, ice crystallization leads to a significant increase in the concentration of IPA (~0.65 mole fraction) in the freeze-concentrate. The plasticizing effect of IPA is evident from the lowering in Tg′ of the system to ~ –29°C (Fig. 2, lower panel).

Low temperature XRD was used to investigate the kinetics of crystallization of cefazolin sodium during annealing. Solutions containing cefazolin sodium (10% w/w) and IPA (5% w/w) were cooled to –40°C at 10°C/min, held for 30 min, and heated to the annealing temperature at 5°C/min. Two annealing temperatures were chosen: –8°C and –15°C. The XRD patterns indicated the crystallization of cefazolin sodium pentahydrate during annealing (Fig. 1c contains the XRD pattern of the sample annealed at –8°C). The kinetics of crystallization was monitored by determining the integrated intensities of the peaks of cefazolin sodium pentahydrate and plotting them as a function of annealing time. For the sake of

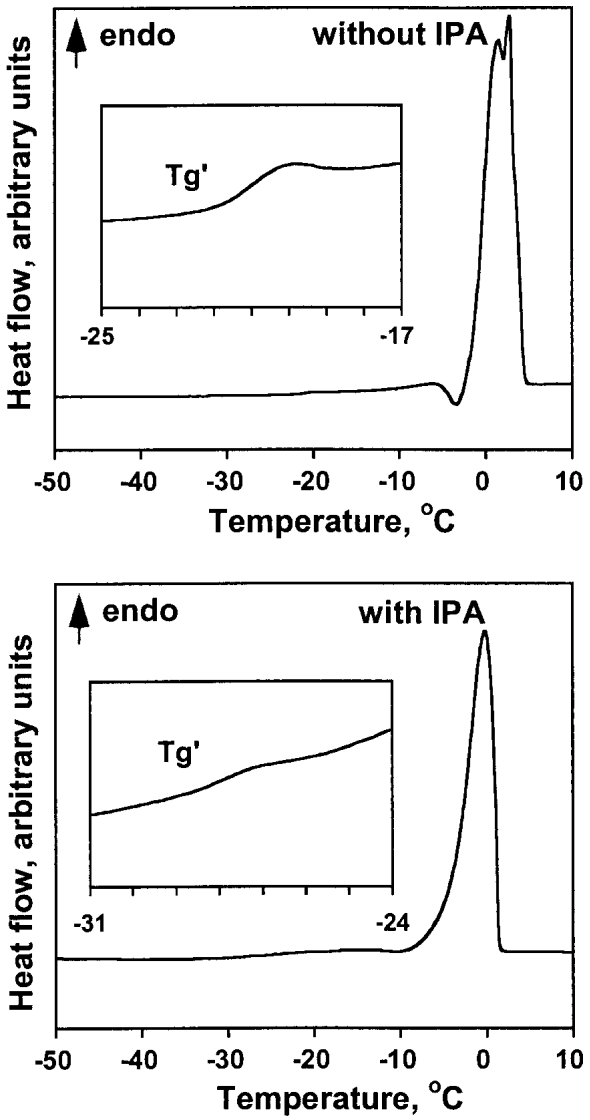

Fig. 2. DSC heating curves of frozen aqueous solutions of cefazolin sodium (10% w/w) in the absence (upper panel) and presence (5% w/w) of IPA (lower panel). The solutions were initially cooled from room temperature to –40°C at 10°C/min, held for 20 min, and heated to room temperature at 5°C/min. Only the heating profiles are shown. Insets show the regions of Tg′.

comparison, the crystallization of cefazolin sodium in the absence of IPA also was monitored at –15°C and has been plotted (Fig. 3). The presence of IPA combined with the higher annealing temperature facilitated the crystallization of cefazolin sodium pentahydrate in frozen aqueous solutions.

The crystallization of cefazolin sodium pentahydrate may have been facilitated by two factors. The first is a lowering of the drug solubility in an alcohol-water mixture. Under ambient conditions, cefazolin sodium is freely soluble in water, whereas in alcohol, it is very slightly soluble (18). This translates to a lower solubility of cefazolin sodium in water-alcohol systems, especially at subambient temperatures. As the temperature is lowered, the IPA content in the system will increase because of ice crystallization. This is expected to have a pronounced effect on the solubility of cefazolin sodium. Secondly, we had earlier observed that in the presence

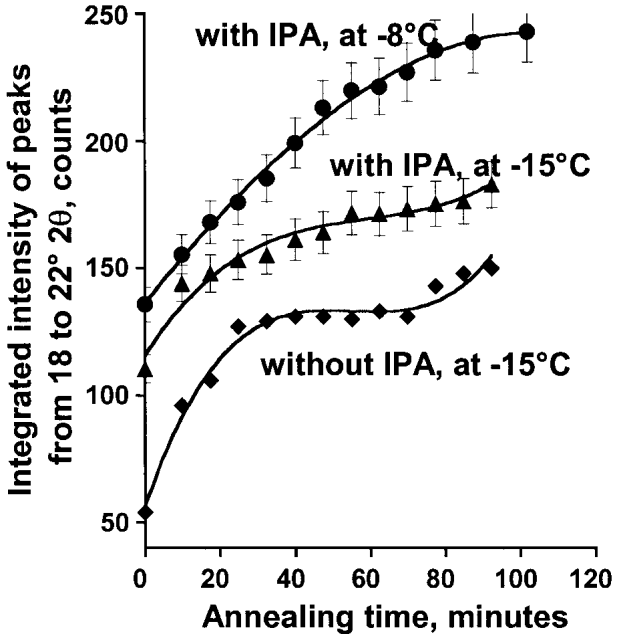

Fig. 3. Integrated intensity of peaks of cefazolin sodium pentahydrate from 18 to 22°2θ (mean ± SD; n = 3) as a function of annealing time: without IPA at –15°C, with IPA (5% w/w) at –15°C, and with IPA (5% w/w) at –8°C. The curves were drawn to assist in visualizing the trends.

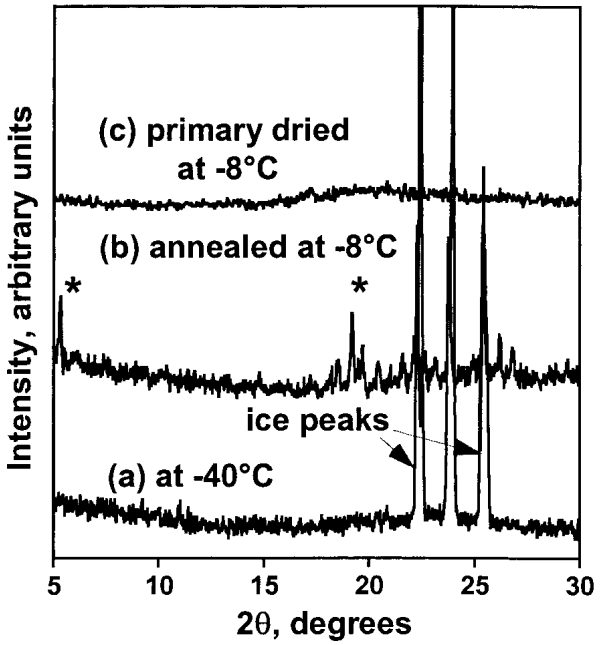

Fig. 4. XRD patterns obtained during freeze-drying of cefazolin sodium (10% w/w). (a) After the solution was cooled from room temperature to –40°C at 10°C/min. (b) It was then heated to –8°C at 5°C/min and annealed for 2 h. The XRD pattern was obtained at the end of annealing. Some of the characteristic peaks of cefazolin sodium pentahydrate are marked with an "*". (c) After primary drying was carried out at –8°C for 2 h.

of IPA, the Tg′ was significantly lowered (Fig. 2). Therefore, at any annealing temperature, the (T-Tg′) value for the frozen solution containing IPA is much higher than that in the absence of IPA. Since the mobility will increase as a function of (T-Tg′), the enhanced mobility of the system containing IPA is expected to accelerate crystallization.

Transitions during Freeze-Drying

The next step in the freeze-drying cycle is primary drying. A prerequisite for obtaining a crystalline lyophile is the crystallization of the active in the frozen state. Frozen aqueous solutions of cefazolin sodium (10% w/w) and IPA (5% w/w) that were annealed at –8°C were subjected to primary drying either at –8 or at –40°C. During primary drying at –8°C, there was a decrease in the intensities of the characteristic peaks of cefazolin sodium pentahydrate (Fig. 4). This was attributed to the dehydration of the pentahydrate to form an X-ray amorphous anhydrous phase in the final product. There also was sublimation of ice. Both events could be monitored simultaneously by plotting the intensity of the characteristic peaks of ice and cefazolin sodium pentahydrate as a function of primary drying time (Fig. 5).

At a primary drying temperature of –8°C, both sublimation of ice and dehydration of the pentahydrate occurred rapidly (Fig. 5, upper panel). These processes appeared to be complete in less than 30 min. To determine the effect of primary drying temperature on the kinetics of these processes, the annealed sample was cooled to –40°C and primary dried. As expected, both sublimation and dehydration were slowed and occurred at a much slower rate than at –8°C (Fig. 5, lower panel). Moreover, at the lower temperature, sublimation preceded dehydration.

Water coexists in three forms during the primary drying stage: ice, water of crystallization (stoichiometric water associated with a hydrate), and unfrozen water in the amorphous freeze-concentrate. The thermodynamic driving force for water removal is directly proportional to the differences in the vapor pressure of water and the chamber pressure. Typically, ice undergoes sublimation even at –40°C because of its vapor pressure, whereas the loss of the lattice water and the unfrozen water would occur at a relatively slower rate at this temperature (19).

In a frozen solution, if the solute crystallizes as a hydrate, primary drying could lead to dehydration and potential loss in crystallinity. For example, when aqueous solutions of sodium nafcillin were annealed, crystallization of "sodium nafcillin hydrate" (hydrate stoichiometry not known) was observed (7,9). Primary drying at –10°C led to partial dehydration to a poorly crystalline sodium nafcillin hemihydrate. In the present case, cefazolin sodium pentahydrate dehydrated readily, especially at higher temperatures.

Effect of Crystalline Bulking Agents

The primary function of a bulking agent in a freeze-dried formulation is to prevent "blow-out" of the dosage form. This is especially true for those systems where the active ingredient is present in very small quantities. Bulking agents are also incorporated in formulations to enhance pharmaceutical elegance and to facilitate drying (5,20). Usually these "fillers" are good cake-formers and extend structural integrity and robustness to the lyophile. Moreover, if the bulking agents crystallize, the system may be subjected to a higher primary drying temperature, as the maximum allowable temperature in the case of crystalline solutes is the eutectic melting tem-

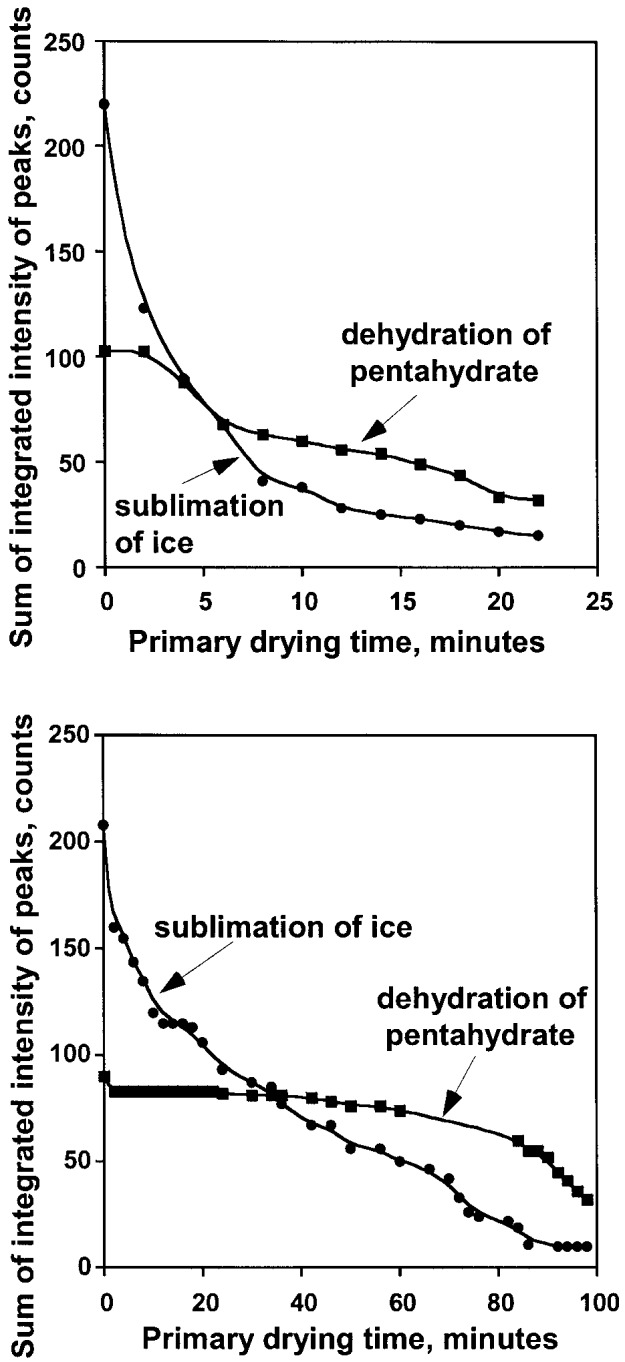

Fig. 5. Sum of the integrated intensities of peaks of cefazolin sodium pentahydrate from 18 to 22°2θ (circles) and of ice from 22 to 26°2θ (squares) as a function of primary drying time at –8°C (upper panel) and at –40°C (lower panel).

perature. On the other hand, if they remain amorphous, they will form a part of the amorphous freeze-concentrate. Amorphous mannitol and glycine, especially the latter, act as plasticizers and tend to lower the Tg′ of the system. To prevent collapse, the primary drying is conducted below the Tg′ of the system. Usually, the Tg′ of an amorphous freeze-concentrate is significantly lower than the eutectic melting temperature of its crystalline counterpart.

Two commonly used crystallizing bulking agents are mannitol and glycine. Glycine (an amino acid) and mannitol (a nonreducing sugar) both crystallize readily in frozen and freeze-dried systems. Additives significantly affect the solid state of the active ingredient during the different stages of the freeze-drying process. It has been reported that the crystallization of cefazolin sodium was inhibited in the presence of saccharides which remained amorphous on freeze-drying (21). Therefore, it was of interest to study the effect of crystallizing bulking agents on the solid state behavior of cefazolin sodium. The hypothesis was that crystallizing bulking agents would facilitate crystallization of the active ingredient. Moreover, as mentioned earlier, amino acids such as glycine and alanine have been shown to increase the crystallinity of freeze-dried drugs.

Transitions in Frozen Aqueous Solutions

In the next set of experiments, the cooling rate was decreased to 2°C/min. When aqueous cefazolin solution (10% w/w), was cooled from room temperature to –70°C in the DSC, in addition to ice crystallization, an exotherm attributable to the crystallization of cefazolin sodium was observed. (Results not shown.) When the same experiment was conducted in the XRD, there was no evidence of solute crystallization, suggesting that this was below the detection limit of the technique.

The effect of glycine (in the concentration range of 0.25–10% w/w) on the solid-state of cefazolin sodium in frozen aqueous solutions was then investigated. In the presence of glycine (0.25% w/w), crystallization of cefazolin sodium was evident in solutions cooled to –70°C, both in the DSC and XRD. Up to a glycine concentration of 5% w/w, while DSC revealed crystallization of cefazolin sodium, XRD did not. Interestingly, on increasing the glycine concentration to 7% w/w, the DSC profiles revealed only ice crystallization. At a glycine concentration of 10% w/w, both glycine and cefazolin sodium pentahydrate crystallized during cooling. These results were also confirmed by low temperature XRD. (Data not shown.) Thus, the crystallization of glycine was evident only when its concentration was 10% w/w.

The heating profiles provided further insight into the possible role of glycine in modifying the solid state of cefazolin sodium. As pointed out earlier, the Tg′ of cefazolin sodium is ~–22°C. The Tg′ of glycine is ~–75°C. Addition of glycine lowered the Tg′ of the system and the temperature of crystallization of cefazolin sodium pentahydrate (Fig. 6, upper panel). At a glycine concentration of 7% w/w, no solute crystallization during cooling was detected. On heating this system, two crystallization exotherms were seen at ~ –20 and at ~ –15°C. Based on low temperature XRD, the first exotherm was attributed to the crystallization of cefazolin sodium pentahydrate and the second to the crystallization of glycine. When the glycine concentration was increased to 10% w/w, no thermal transitions were observed over the temperature range of –50 to –10°C. In this case, cefazolin sodium crystallized during cooling, as confirmed by low temperature XRD. Glycine facilitated the crystallization of cefazolin sodium pentahydrate. This conclusion is based on two observations: (i) with an increase in concentration of glycine, the temperature of crystallization was lowered; and (ii) at the highest concentration of glycine, both glycine and cefazolin sodium crystallized during the cooling step.

To further investigate the effects of glycine on the crys-

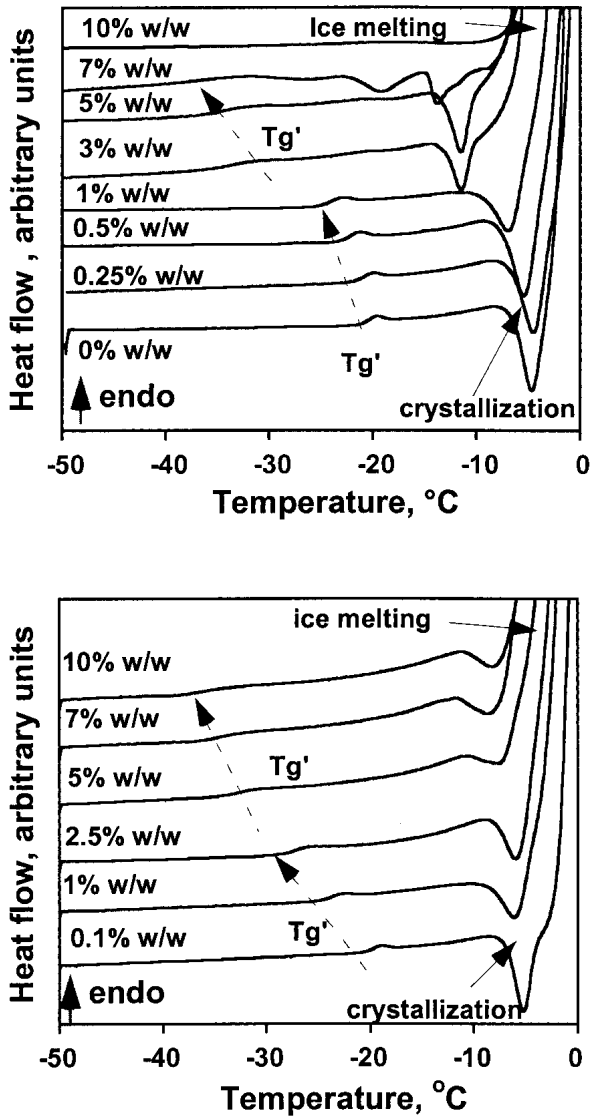

Fig. 6. Upper panel. DSC heating profiles of frozen aqueous solutions containing cefazolin sodium (10% w/w) and glycine. The solutions were initially cooled from room temperature (RT) to –70°C at 2°C/min and heated back to RT at 5°C/min. Lower panel. DSC heating profiles of frozen aqueous solutions containing cefazolin sodium (10% w/w) and mannitol. The solutions were cooled from RT to –50°C at 2°C/min and heated back to RT at 5°C/min. The additive (mannitol or glycine) concentration in the solution is given above each of the DSC curves.

tallization behavior of cefazolin sodium, the frozen solution was studied isothermally using low temperature XRD. Aqueous solution of cefazolin sodium (10% w/w) was cooled at 20°C/min from RT to –50°C, held for 30 min and heated to –20°C at 5°C/min, and annealed at this temperature for 1 h. No solute crystallization was detected. When a similar experiment was carried out in the presence of 0.25% w/w glycine, peaks of cefazolin sodium pentahydrate were observed. (Data not shown.) These studies revealed that the presence of glycine, even at a low concentration, facilitated the crystallization of cefazolin sodium pentahydrate in frozen aqueous solutions.

On the other hand, mannitol affected the crystallization of cefazolin sodium in a significantly different manner. We studied the effect of mannitol concentration in the range of 0.1–10% w/w. The cooling profiles (solutions cooled from room temperature to –50°C at 2°C/min) did not show very reproducible behavior. However, the DSC heating curves of these solutions provided useful information (Fig. 6, lower panel). As the concentration of mannitol was increased from 0.1–10% w/w, the Tg′ of the system was lowered from –22°C to –39°C. The next event was the crystallization exotherm of cefazolin sodium. While the mannitol concentration did not significantly alter the temperature of crystallization, the enthalpy of crystallization decreased with an increase in the concentration of mannitol. Thus, mannitol might be exhibiting a concentration-dependent inhibition in the crystallization of cefazolin sodium pentahydrate.

We believe that in the presence of cefazolin sodium, mannitol crystallization was inhibited, which in turn, inhibited the crystallization of the active ingredient. The degree of inhibition was proportional to the amorphous mannitol concentration, which increased as a function of the total mannitol concentration. The Tg′ values gave us an indirect proof. The mannitol-water system is characterized by two glass transition temperatures at –25 and at –32°C (22). At mannitol concentrations > 5% w/w, the Tg′ of the system was not intermediate between the Tg′ values of the individual components. That is, the Tg′ was lower than that of mannitol (–25 and –32°C) as well as cefazolin sodium (–22°C).

One explanation could be that by remaining amorphous, mannitol forms part of the amorphous freeze-concentrate. This probably increases the amount of unfrozen water in the system, thereby lowering the Tg′ of the system. Similar behavior in the presence of mannitol has been documented earlier in the literature, wherein the Tg′ of a system containing mannitol and sucrose was lower than that of any of the individual components (23).

Low temperature XRD studies provided more direct evidence of the effect of mannitol. Mannitol prevented the crystallization of cefazolin sodium in frozen aqueous solutions. This conclusion is based on the following observations. (i) On cooling (10% w/w) to –50°C at 2°C/min, aqueous solutions of mannitol usually crystallize. However, mannitol did not crystallize when aqueous solutions of cefazolin sodium (10% w/w) and mannitol (0.1–10% w/w) were cooled to –50°C at 2°C/min. We compared the intensities of cefazolin sodium peaks after annealing at –10°C in the presence of 0.1% w/w and 10% w/w mannitol. The amount crystallized (from the integrated intensity of the peaks of cefazolin sodium pentahydrate) and as well as its crystallinity (from the full width at half maxima of the peaks of cefazolin sodium pentahydrate) was lower when the mannitol concentration was higher. (ii) At the beginning of annealing, the amount of crystalline cefazolin sodium pentahydrate formed was lower when the mannitol concentration was higher.

Transitions during Freeze-Drying

The freeze-drying of aqueous solutions of mannitol alone led to the formation of the crystalline dehydrated hydrate while in the case of glycine, the anhydrous β-polymorph was obtained.

If freeze-drying of the drug and excipient was carried out without an annealing step in the frozen state, the lyophile

contained either β-glycine or δ-mannitol depending on the crystallizing cosolute that was present (Fig. 7). Cephazolin sodium was X-ray amorphous in the final product. This showed that the mere presence of crystallizing cosolutes did not result in a crystalline active ingredient in the lyophile.

The next step was to incorporate an annealing step in the frozen system. Aqueous solution containing cefazolin sodium (10% w/w) and glycine (10% w/w) was cooled to –70°C and then heated to –10°C where it was annealed for 2 h. While there was no solute crystallization on cooling (Fig. 8, profile [a] in the upper panel), annealing led to the crystallization of cefazolin sodium pentahydrate and β-glycine (Fig. 8, profile [b] in the upper panel). The crystallinity of cefazolin sodium was retained at the end of the freeze-drying process after primary drying at –10°C (Fig. 8, profile [c] in the upper panel). In the absence of glycine, primary drying resulted in an X-ray amorphous lyophile (Fig. 4, profile [c]). Thus, crystalline cefazolin sodium was obtained only in the presence of glycine.

The XRD pattern of cefazolin sodium in the final product matched that of the crystalline pentahydrate. Thermogravimetric analysis revealed a water content of ~7.5% w/w of the lyophile. The lyophilized solution contained 10% w/w of each cefazolin sodium and glycine. If we assume that all the water was associated with cefazolin sodium, then the observed water content is close to the stoichiometric water content of ~15% in cefazolin sodium pentahydrate ($C_{14}H_{13}N_8O_4S_3Na \cdot 5H_20$). However, since the final product is not completely crystalline and amorphous cefazolin sodium is highly hygroscopic, it is expected that some of the residual water would be associated with the noncrystalline phase. Therefore, the cefazolin sodium is likely to exist as a mixture of cefazolin sodium pentahydrate and X-ray amorphous anhydrous cefazolin containing sorbed water. At the end of primary drying, when the secondary drying was carried out at 25°C, there was a decrease in the intensity of the peaks of cefazolin sodium pentahydrate, suggesting that the system underwent dehydration at the higher temperature.

According to Korey and Schwartz, the possible reasons

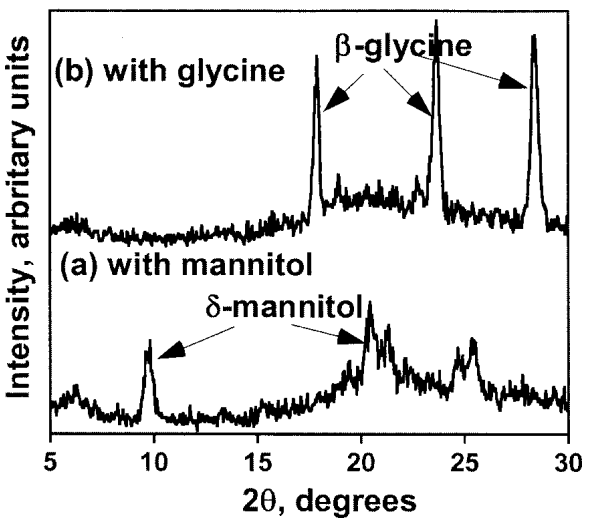

Fig. 7. XRD patterns of freeze-dried cefazolin sodium (10% w/w) in the presence of (a) mannitol (10% w/w) and (b) glycine (10% w/w). The samples were freeze-dried at –10°C without any annealing. Cephazolin sodium existed in the X-ray amorphous state in the lyophile.

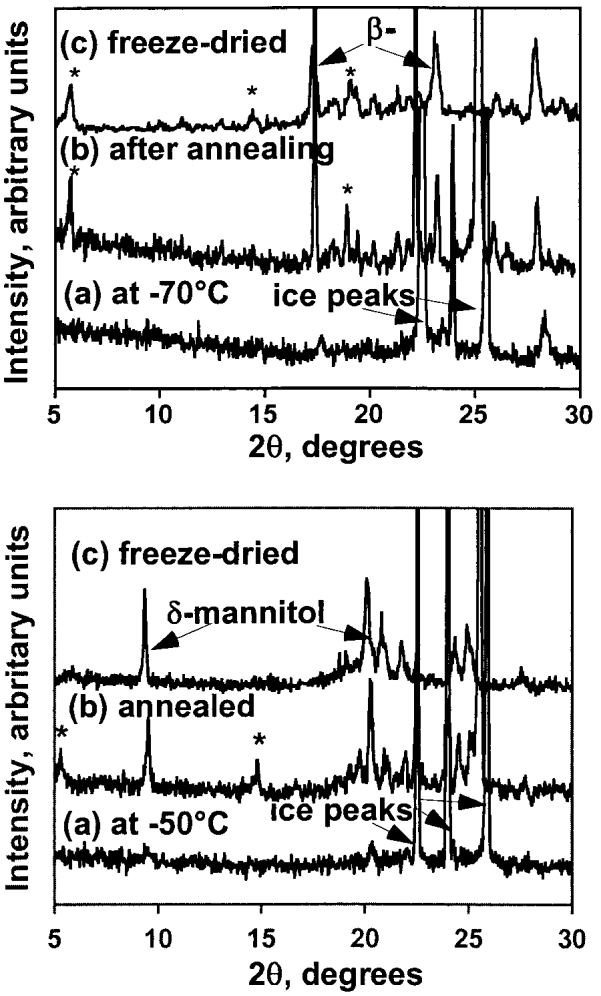

Fig. 8. Upper panel: XRD patterns obtained during freeze-drying of cefazolin sodium (10% w/w) in the presence of glycine (10% w/w) (a). Solution cooled from room temperature to –70°C at 2°C/min (b). Heated to –10°C at 5°C/min and annealed for 2 h. Annealing led to the crystallization of cefazolin sodium pentahydrate (peaks marked with an "*") and β-glycine (c) after primary drying at –10°C for 2 h. Lower panel: XRD patterns obtained during freeze-drying of cefazolin sodium (10% w/w) in the presence of mannitol (10% w/w) (a). Solution cooled from room temperature to –50°C at 2°C/mi (b), heated to –10°C at 5°C/min and annealed for 2 h. Annealing led to the crystallization of cefazolin sodium pentahydrate (peaks marked with an "*") and δ-mannitol (c) after primary drying at –10°C for 2 h.

for the facilitation of crystallization of cefazolin sodium by glycine were: (i) formation of a complex between the excipient and the active, (ii) the excipient's acting as seed crystals, and (iii) the formation of a eutectic (12). Though the mechanism by which glycine can retain cefazolin sodium crystalline during primary drying is still unclear, our results strongly suggest that no crystalline complex is formed during the freeze-drying. The formation of such a complex is expected to result in the appearance of new XRD peaks in the powder pattern. Glycine crystals may have acted as seeds, since amino acids have been used as "templates" for enhancing crystallization of compounds (24).

On the other hand, mannitol did not affect the solid-state behavior of cefazolin sodium during drying. Two concentrations of mannitol were studied: 10% and 5%. As observed

earlier, on annealing frozen aqueous solutions containing cefazolin sodium and mannitol (Fig. 8, profile [a] in the lower panel), both the solutes crystallized, with the former crystallizing as the pentahydrate (profile [b]). At both mannitol concentrations, primary drying at –10°C led to the dehydration of the pentahydrate and formation of the X-ray amorphous phase in the final product (profile [c]). Mannitol crystallized as the anhydrous δ-mannitol. *In situ* XRD thus revealed that the presence of mannitol did not retain cefazolin sodium in the crystalline state during primary drying.

When mannitol solutions were freeze-dried under similar conditions, crystallization of mannitol hydrate often was observed (7). However, in the presence of cefazolin sodium, only the anhydrous δ-polymorph was obtained (Fig. 8; lower panel). Since the pKa of the drug is 2.15, it will be ionized almost completely under all the experimental conditions of the current study (25). The influence of ions on the solid phase of mannitol crystallizing from solution has not been investigated, and will be the subject of another study.

The phase transitions that may be observed during freeze-drying are: (i) crystallization of an amorphous phase, (ii) polymorphic transitions, and (iii) dehydration of a hydrate. If the primary concern of the formulator is to obtain a crystalline active ingredient in the final product, special attention is desired in the case of compounds that can exist as hydrates. In these cases, as was seen in the present study, a hydrate might crystallize during annealing of the frozen aqueous solutions. However, during the primary drying, dehydration may occur, resulting is an amorphous anhydrate. This, again, is expected to be a function of the processing conditions and formulation variables. Thus, it is important to characterize a system during all the stages of freeze-drying to obtain information that will assist in the design of a rational freeze-drying cycle.

Evaluation of Strategies to Crystallize Drug Molecules during Freeze-Drying

Among the strategies commonly used to obtain crystalline lyophiles, the present work has focused on the effects of annealing of frozen aqueous solutions and the role of organic cosolvents and crystalline bulking agents. The effect of solution cooling rate on the crystallization of cefazolin sodium also was investigated, though the details are not presented. During cooling at rates ≤ 1°C/min, partial crystallization of cefazolin sodium was observed. However, the amount of crystalline cefazolin sodium at the end of annealing appeared to be independent of the cooling rate. Therefore, we chose to use rapid cooling rates. However, slower cooling rates will facilitate more-complete ice crystallization, and this may aid crystallization of the solute. Therefore, one should not rule out the potential utility of slower cooling rates in promoting solute crystallization.

Ice crystallization also is known to be influenced by organic cosolvents (26). While we have extensively discussed the use of IPA, tertiary butyl alcohol (TBA) is a cosolvent that tends to crystallize in frozen systems. TBA is known to modify the crystal habit of ice and promote its sublimation (26,27). However, TBA crystallizes readily as tertiary butyl alcohol dihydrate in frozen aqueous solutions. The thermal behavior of TBA-water systems is complex. Kasraian and Deluca reported the formation of two eutectics, one at 20% and the other at 90% w/w TBA (28). The eutectic melting temperatures were ~–5 and –3°C respectively. On the other hand, the IPA-water phase diagram indicated that IPA crystallizes in frozen aqueous solutions only at temperatures as low as –90°C (17). Thus, IPA is present as a liquid at temperatures that are practically relevant in freeze-drying. As mentioned before, IPA affects the Tg′ of the system. When TBA crystallizes in frozen aqueous solutions, it does not alter the Tg′ of the residual amorphous phase. Kasraian and Deluca also reported that TBA did not modify the collapse temperature (which is practically coincidental with Tg′) of sucrose (26). Based on the present work, it is evident that IPA is incorporated in the amorphous freeze-concentrate. It therefore can modify the solubility of cefazolin sodium and the Tg′ of the system, thereby facilitating solute crystallization. Finally, this work reveals the importance of an annealing step for solutes that do not crystallize readily. This can ensure consistent solute crystallization and minimize batch-to-batch variability.

CONCLUSIONS

The phase transitions during cooling, in the frozen state, and during the drying of aqueous cefazolin sodium solution were investigated as a function of the processing conditions and formulation variables. The physical state of solutes in freeze-dried systems depends on a complex interplay of formulation and processing conditions. This study reveals the importance of physical characterization during all stages of the freeze-drying process, as crystallization of a solute in the frozen state did not ensure a crystalline lyophile. Though cefazolin sodium crystallized as the pentahydrate in frozen aqueous solutions, dehydration during drying led to the formation of an X-ray amorphous lyophile. However, in the presence of glycine, the active ingredient was retained in the crystalline state at the end of the freeze-drying cycle.

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