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Research Paper

Crystallization of Cephalothin Sodium During Lyophilization from *tert*-Butyl Alcohol–Water Cosolvent System

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Purpose. Because cephalothin sodium (**I**) does not crystallize readily when freeze-dried from aqueous solutions, organic cosolvents were used to increase the crystallinity of lyophilized **I**.
Methods. Compound **I** was lyophilized from water-organic cosolvent (5% w/w) systems of each ethanol, ispropanol, and *tert*-butyl alcohol (TBA).
Results. When frozen solutions of **I** (10% w/w) in each of these cosolvent systems was characterized by DSC, the presence of cosolvent in the freeze-concentrate was evident. Moreover, the presence of the cosolvent accelerated the solute crystallization. This observation was based on the XRD of these systems during the various stages of freeze-drying. High initial solute concentration and annealing of frozen solutions facilitated the formation of a highly crystalline lyophile. The accelerated crystallization is attributed to supersaturation in cosolvent systems, facilitating nucleation during freezing with subsequent growth during annealing. Lyophiles obtained from water-isopropanol and water-ethanol systems collapsed, while the use of TBA as a cosolvent yielded a friable and pharmaceutically elegant cake, containing fine needle-shaped crystals of **I**. Gas chromatography revealed a residual TBA concentration of ~0.001% w/w in the crystalline lyophiles. In general, residual cosolvent levels were higher in lyophiles with lower crystallinity.
Conclusions. TBA-water was found to be a suitable freeze-drying medium to promote crystallization of **I** and yielded a lyophile with desirable product characteristics.

KEY WORDS: cephalothin; crystallinity; freeze-drying; *tert*-butyl alcohol; X-ray diffractometry.

INTRODUCTION

Lyophilization is widely used for the preparation of parenteral dosage forms and allows for the preservation and stabilization of thermolabile drugs and biologicals that have limited shelf-life in solutions. Although some buffer salts and bulking agents are known to be crystalline at the end of the freeze-drying cycle, lyophiles are generally amorphous as is seen for proteins and sugars. It is well recognized, however, that amorphous products may be both physically and chemically less stable than their crystalline counterparts (1).

Given that water is commonly the solvent of choice, lyophilization is usually restricted to drugs that are soluble and stable in aqueous media. However, freeze-drying from water-organic solvent (hereafter referred to as "cosolvent") systems has also been successfully accomplished (2–4). Recently, Teagarden and Baker have presented an excellent review on the practical aspects of using non-aqueous cosolvents in lyophilization (5). They provide an exhaustive list of examples from research laboratories as well as commercially manufactured products freeze-dried from aqueous-organic cosolvent systems. Among the advantages conferred have been increased solubility of hydrophobic drugs in the prelyo solution, (6) decreased drying time, (2,7) improved product stability (8,9) and reconstitution characteristics (10,11). The incorporation of isopropanol (IPA) as a colvent increased the crystallinity of cefazolin sodium in the lyophile (12).

Among non-aqueous solvents, tertiary butyl alcohol (TBA) is of particular interest because of its high freezing and eutectic temperatures (13,14). In presence of TBA, ice crystals are needle shaped, thereby decreasing resistance to vapor flow and accelerating sublimation (2). However, the role of TBA in increasing the crystallinity of drugs has not been explored. Ni *et al.* obtained needle shaped drug crystals after lyophilizing an antitumor drug from pure TBA (9), while "erratic" crystallization of lactose from a TBA-water formulation during lyophilization has been reported (7).

Cephalothin sodium (**I**), a cephalosporin antibiotic, was chosen as the model compound due to its propensity to remain amorphous after freeze-drying. This amorphous form discolors rapidly. While partially crystalline samples have been prepared by annealing frozen solutions for as long as 18 h (1), addition of sterile seed crystals (15,16) have induced crystallization as well. These established means of causing crystallization have been successful in obtaining a crystalline powder of **I** (17).

Our objective was to develop a mechanistic understanding of the influence of TBA in increasing the crystallinity of **I**.

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In an earlier study, the use of isopropanol as a cosolvent promoted the crystallization of cefazolin sodium in frozen aqueous systems (12). This was attributed to a decrease in glass transition temperature brought about by the cosolvent (18). Given the high freezing point of TBA, such a mechanism seems unlikely. We base the objective of the current study on the reduced solubility of cephalothin sodium in TBA. To the best of our knowledge, TBA has not been included in lyophilized formulations of **I** (19,20). While we wanted to explore the potential ability of TBA-water systems to yield crystalline lyophiles and compare such lyophiles with those obtained from water as well as other commonly used aqueous-cosolvent mixtures, it was also of interest to evaluate the lyophile characteristics including elegance, structural features and residual cosolvent levels.

MATERIALS AND METHODS

Materials

Cephalothin sodium (**I**) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvents used were *tert*-butyl alcohol (GC grade, Fischer, Fairlawn, NJ, USA), isopropyl alcohol (HPLC grade, Sigma), ethanol (HPLC grade, Sigma) and *n*-butanol (GC grade, Fischer).

Differential Scanning Calorimetry (DSC)

A differential scanning calorimeter (model 2920, TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system was used. Mercury was used for sub-ambient temperature calibration. About 10–15 mg of the solutions were weighed in aluminum pans and hermetically sealed. Samples were cooled from room temperature to –70°C at 1°C/min, held for 15 min, and warmed at 2°C/min to 25°C. Details of annealing treatments are in the Results and Discussion section.

X-Ray Diffractometry (XRD)

Low-Temperature XRD

An x-ray powder diffractometer (Scintag model XDS 2000, Sunnyvale, CA, USA) with a variable temperature stage (Micristar, model 828D; working temperature range of –190°C to 300°C) was used. One hundred milligrams of samples were accurately weighed into a copper sample holder. Specific experimental details are provided in the Results and Discussion Section. XRD patterns were obtained with Cu Kα radiation (45 kV × 40 mA) at a scanning speed of 5°2θ/min and a step size of 0.03°2θ. During the scan, the sample was maintained at a constant temperature.

In Situ Freeze-Drying

This was performed by attaching a vacuum pump (pressure ~100 mTorr) to the temperature stage of the diffractometer (Scintag model XDS 2000), enabling the freeze-drying cycle to be carried out in the sample chamber of the XRD.

Crystallinity Determination

Mixtures of amorphous (**I** lyophilized from water, no annealing treatment) and crystalline (commercially available) **I** were prepared by geometric dilution under low RH conditions (~0%). The water content in the samples was negligible. Samples were filled in an Al holder by the side-drift method and exposed to Cu Kα radiation (45 kV and 40 mA) in a wide-angle x-ray diffractometer (model D5005, Siemens). The instrument was operated in the step-scan mode, in increments of 0.01°2θ. The angular range was 8.9 to 10.5°2θ and counts were accumulated for 1.5 s at each step. The integrated intensity of the 9.6°2θ peak of **I** was obtained after appropriate background subtraction. The data collection and analyses were performed with commercially available software (JADE, version 3.1, Materials Data, Inc, Livermore, CA, USA). A plot of the peak intensity as a function of the % crystallinity of **I** yielded a linear relationship. The equation of the line was:

$$\text{Peak intensity} = 139.2 \times \%\ \text{crystallinity} - 561.7 \quad (r^2 = 0.974)$$

Freeze-Drying

Solutions of **I** were prepared at concentrations of 10% and 20% w/w in aqueous-cosolvent solutions, with cosolvent concentrations of 5% and 10% w/w. Aqueous drug solutions served as controls. These were passed through 0.2 μm filters, filled into 5 ml borosilicate serum vials (3 ml fill volume), and transferred to a laboratory freeze–dryer (Virtis Advantage, benchtop, Gardiner, NY, USA) equipped with an organic solvent trap (Virtis). Vials were covered with gray butyl split rubber stoppers (VWR, West Chester, PA, USA). Samples were cooled to a shelf temperature of –55°C for 6 h, followed by primary drying at –15°C for 40 h (80 mTorr chamber pressure). Selected samples were annealed at –15°C for 4 h prior to primary drying. Secondary drying was conducted at a shelf temperature of 25°C for 30 h. The heating and cooling rates were 0.5°C/min.

Quantification of Residual Solvent in the Lyophile

The residual organic solvent content in the lyophiles was quantified by a gas chromatographic (GC) method (21) with a carbowax column (15 × 0.25 mm, Supelco Inc., Bellefonte, PA, USA), and a flame ionization detection system (Shimadzu, Model GC-8). The column and injector temperatures were 100 and 150°C respectively. Freeze-dried solids were weighed (50 mg) and reconstituted with 2 ml of distilled water. The internal standard was n-butanol.

Scanning Electron Microscopy

The lyophile powder samples were mounted on SEM stubs with double-sided carbon tape. Platinum coated samples (50 Å) were observed under a scanning electron microscope (Hitachi S-800, Tokyo, Japan).

RESULTS AND DISCUSSION

Characterization of I

Compound **I** is commercially available as a crystalline anhydrate and its XRD pattern matches that in the Powder Diffraction Files of the International Center for Diffraction Data (22). When heated in the DSC, **I** decomposes near its melting temperature. There are no reports of hydrates or polymorphic forms of the anhydrate. The water content, determined by Karl Fischer titrimetry, was <0.5% w/w.

Characterization of Frozen Solutions

Because freezing, followed by rewarming are usually the first steps of lyophilization, it was of interest to monitor the thermal events in these stages. The DSC profiles of different water-cosolvent frozen solutions are overlaid in Fig. 1. The Tg' of the aqueous solution of **I** was –23°C (Fig. 1, curve i) in agreement with that reported earlier (23). This was followed by an ice melting endotherm with onset at ~ –5°C. The presence of cosolvent caused a distinct drop in Tg' of the solute as is evident from the inset of Fig. 1. No Tg' was detected in the ethanol–water freeze-concentrate. Because the Tg of ethanol is –175.9°C (24), the Tg' is expected to be below the temperature range of the experiment (Fig. 1, curve iii). The eutectic temperatures of isopropyl alcohol (IPA)-water and ethanol-water have been reported to be –93.4°C and –121.9°C, respectively (13). It is therefore expected that such solvents will form a part of the amorphous freeze-concentrate, as was seen with IPA and cefazolin sodium (18). *tert*-butyl alcohol (TBA) on the other hand, forms two eutectics with water at TBA concentrations of 20% w/w (TBA hydrate-ice) and 90% w/w (TBA hydrate-TBA), melting at –5°C and –3°C, respectively (13,14). Thus, at the concentrations used here (≤10% w/w), TBA is expected to separate from the amorphous phase as a eutectic of TBA hydrate and ice. However, lowering of Tg' in the TBA system, suggests that a fraction of it is in the amorphous freeze-concentrate. The presence of an amorphous cosolute, for example a sugar, invariably inhibits the crystallization of TBA during cooling (7,21). As a result, an exothermic crystallization event, attributed to TBA crystallization, was observed during warming (Fig. 1, curve iv). Given the extremely low tendency of the model drug to crystallize from aqueous solutions, the exotherm cannot be attributed to phase separation of the drug.

Though TBA crystallized during cooling, its complete crystallization was hindered even at cooling rates as low as 1°C/min (data not shown). In an effort to induce complete crystallization of TBA, a thermal cycling step was introduced wherein the frozen sample was heated to –20°C (just above

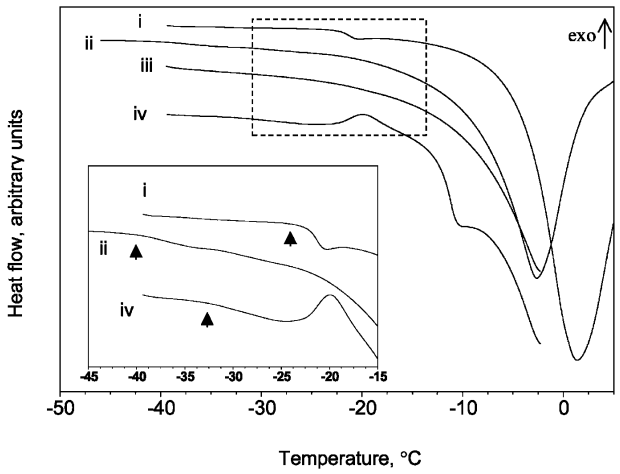

Fig. 1. DSC heating curves (2°C/min) of frozen solutions of **I** (20% w/w) in (i) water and water-cosolvent systems (curves ii–iv). The cosolvents were (ii) isopropanol (IPA) (iii) ethanol, and (iv) *tert*-butyl alcohol (TBA) at a concentration of 5% w/w. Inset: Lowering of Tg' of freeze-concentrate in presence of cosolvents. Arrows point the onset of Tg'.

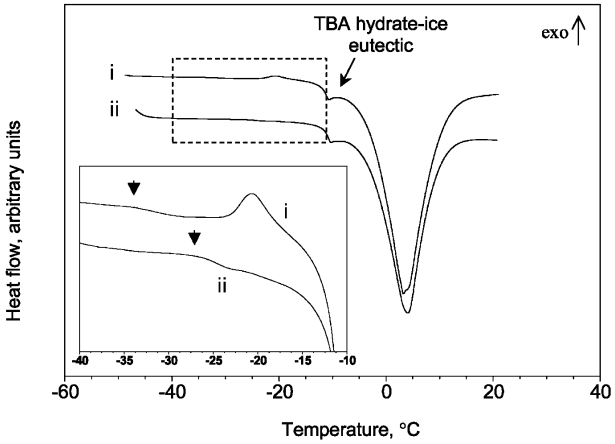

Fig. 2. DSC heating (2°C/min) curves of frozen solutions of **I** (20% w/w) in TBA-water (10% w/w TBA). The solutions were initially cooled from room temperature to –40°C at 5°C/min and held for 10 min. One of the samples (ii) was thermally cycled by heating to –20°C followed by recooling, both at 5°C/min. Final heating profiles are overlaid. The glass transition region has been expanded. Arrows point the onset of Tg'.

Tg'), cooled and then rewarmed (Fig. 2). Even this gentle thermal treatment was adequate to cause complete crystallization of TBA, as evidenced by the absence of the crystallization exotherm and a resultant increase in the Tg' of the system [curve (ii) in Fig. 2]. The physical state of the solute influences TBA crystallization and solutes that remain in the freeze-concentrate hinder its crystallization. The threshold TBA concentration for its crystallization from frozen sucrose solutions was found to be 2% w/w, while from frozen glycine solutions, it was only 0.5% w/w (21). The TBA concentrations in this study (5% and 10% w/w) are high enough to cause its crystallization, despite the amorphous state of the solute.

While ethanol, IPA and TBA have all been used as cosolvents in lyophilized parenteral formulations, some studies suggest that, due to its propensity to crystallize completely, the residual level of TBA is likely to be very low in freeze-dried formulations. This has been confirmed in lyophiles of glycine, sucrose, tobramycin sulfate, SarCNU, an antitumor drug and alprostadil (9,11,21,25). Therefore, we focused on the use of TBA as a cosolvent.

In situ freeze-drying allows phase transitions such as crystallization, sublimation, melting and dehydration to be monitored during various stages of the process (26). In Fig. 3, crystallization of the drug after freezing, annealing and primary drying from TBA-water solvent system is compared with that from water alone. At the end of the freezing step, while only ice peaks were seen in the aqueous drug solution (top), peaks attributable to crystalline drug were discernable in presence of TBA. At a TBA concentration of 10% w/w, while its crystallization was readily evident in the DSC (Figs. 1 and 2), peaks attributable to TBA were not detected in the XRD pattern (Fig. 3). This suggests that XRD is a less sensitive indicator of TBA crystallization than DSC. In an effort to detect crystalline TBA, a 60% w/w TBA solution was cooled to –50°C and subjected to XRD. Based on the phase diagram (Fig. 4, right panel), a mixture of TBA hydrate and ice is expected. The XRD pattern of anhydrous TBA, cooled

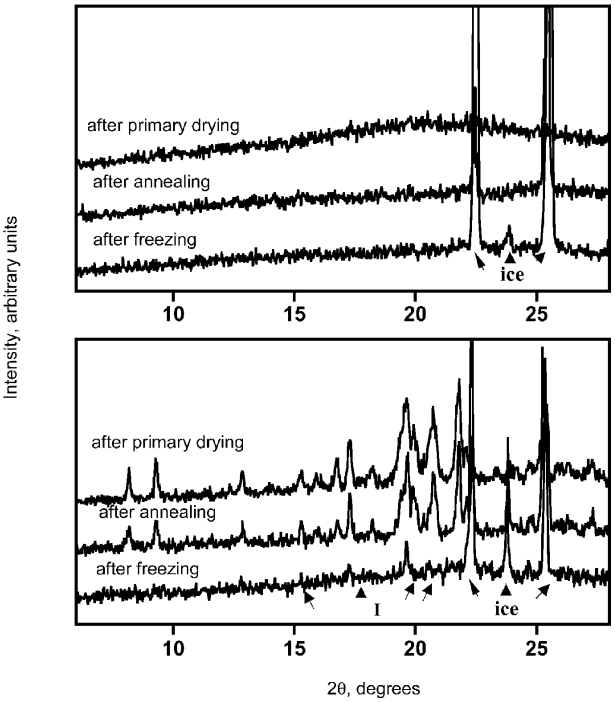

Fig. 3. XRD patterns obtained during freeze-drying of **I** (20% w/w) from aqueous (top) and TBA (10% w/w)-water (bottom) systems. Solutions were cooled to –40°C, held for an hour, warmed to –15°C and annealed for 2 h, followed by primary drying for 4 h at –10°C. Patterns were obtained after three stages of freeze-drying as indicated. Only ice peaks (dark arrows) were seen after freezing the aqueous solution (top), while peaks of **I** were clearly discernable (light arrows) in the TBA-water system (bottom).

to –50°C, was also obtained (Fig. 4, left panel). The XRD pattern of 60% w/w TBA solution is different from that of anhydrous TBA. This strongly suggests crystallization of TBA as a hydrate from the solution.

The absence of crystalline **I** from a frozen aqueous solution (Fig. 3, upper panel), suggests that the rate-limiting step for solute crystallization is nucleation during freezing. When a solution of **I** (20% w/w) in the presence or absence of cosolvent is cooled, a supersaturated state is achieved when water separates as ice. However, given the low solubility of **I** in the three alcohols, the degree of supersaturation is dramatically higher in the cosolvent systems. The solubility of **I** in water (>20 mg/ml at room temperature) is much higher than that in IPA (0.082 mg/ml) or ethanol (1.78 mg/ml) (27). The solubility in TBA at room temperature was estimated to be 0.07 mg/ml.

It is well known that annealing facilitates crystallization. The frozen solutions were annealed at –15°C. While this was above the Tg', it was sufficiently below the temperature at which ice melting began (Fig. 2). The crystallization kinetics of **I** was monitored by low temperature XRD. In Fig. 5, the integrated intensity in the range 19 to 21° 2θ (encompassing many peaks of **I**) was plotted as a function of the annealing time. The peak intensity, and therefore crystallinity, was consistently higher in the presence of TBA, even before annealing was initiated. There was no evidence of solute crystallization in the aqueous system even after annealing for 2 h (see also Fig. 3). On the other hand, in presence of TBA, annealing resulted in pronounced solute crystallization, which translated to a crystalline lyophile (Fig. 6).

If crystallization of the drug were driven mainly by enhanced mobility of the freeze-concentrate as a result of lowered Tg', the effect of TBA-water solvent system in crystallizing **I** would be minimal. The separation of TBA as a TBA hydrate-ice eutectic yields an aqueous freeze concentrate of constant composition, reflected by a fixed Tg' value of –26°C (Fig. 2). In spite of the phase separation of TBA from the freeze-concentrate, a highly crystalline lyophile was obtained as long as the initial solute concentration was high (i.e., 20% w/w) and the system was annealed. Although the TBA-water phase diagram indicates eutectic crystallization at –5°C, our

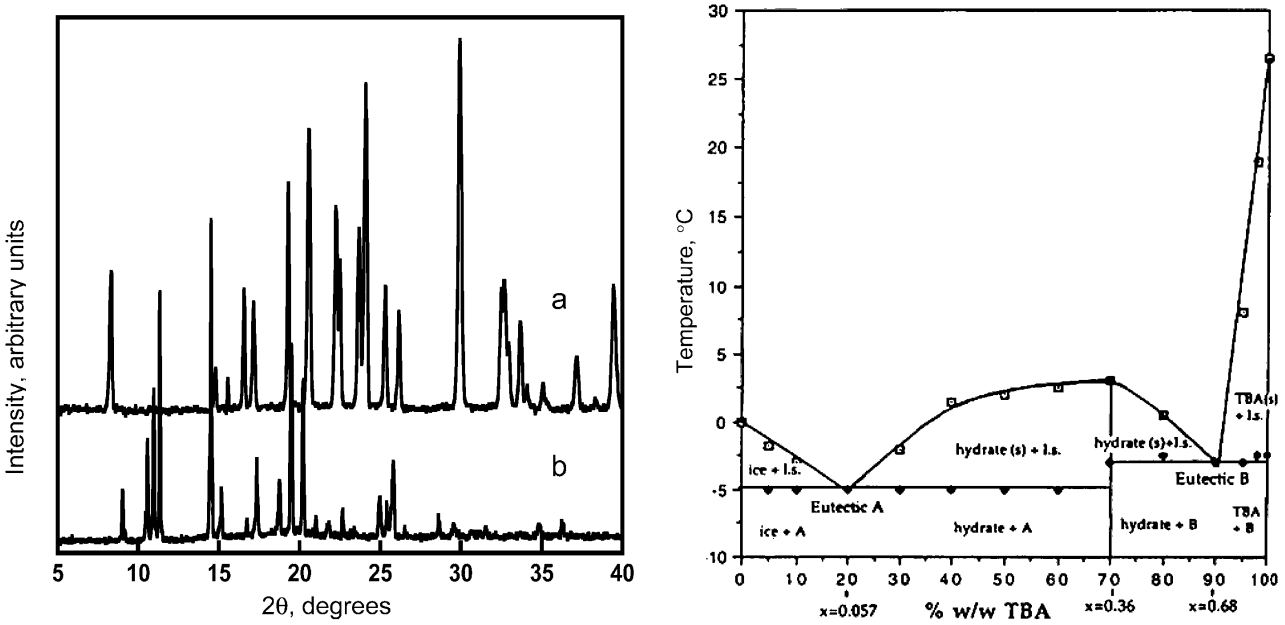

Fig. 4. Left: XRD patterns of (a) TBA and (b) TBA hydrate. Crystalline TBA was not detected at the concentrations used in lyophilization (Fig. 3). Right: Phase diagram of TBA-water, ref 14, reproduced with permission of the copyright owner.

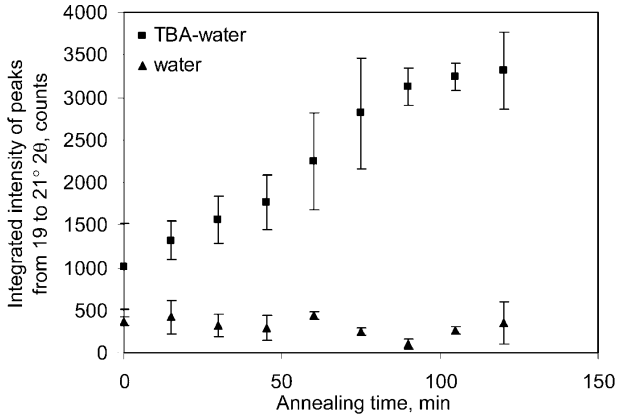

Fig. 5. Crystallization kinetics during thermal treatment of frozen solutions of **I**. Integrated intensity* from 19.0 to 21.1°2θ (mean ± SD; n = 3) are plotted during the first 2 h of annealing. Solutions of **I** (20% w/w) were cooled to −50°C, warmed to −15°C and annealed. The ramping rates were 1°C/min. (*The background counts (~300) were not subtracted.)

DSC experiments revealed that the crystallization of TBA was inhibited, possibly by the amorphous **I**, until the system was cycled or annealed. When lyophilization is initiated and the solution is cooled, ice crystallizes and the concentration of TBA in the freeze-concentrate undergoes a dramatic increase. Because the solubility of **I** in this freeze-concentrate will be much less than that in water, the system is highly supersaturated thereby facilitating nucleation. Though TBA eventually crystallizes from the freeze-concentrate, the solute seeds remain and, on annealing, result in a substantially crystalline lyophile. Thus, the TBA in the freeze-concentrate appears to be responsible for the solute nucleation. When the TBA concentration was decreased to 5% w/w, a highly crystalline lyophile was obtained (Table I). This is because the composition of the freeze-concentrate is independent of the initial solution composition, as reflected by a Tg' value very close to the Tg' value of −34°C of the 10% w/w TBA solution (Fig. 2) and the supersaturation is still high to favor drug crystallization. However, there is a limit to the degree of supersaturation that is necessary for crystallization from this cosolvent system. With 2% w/w TBA, an amorphous lyophile was obtained (not shown). Correlation of residual solvent levels with initial TBA content is well established (21). It is in fact likely that low TBA concentrations will limit its crystallization and therefore hinder sublimation, leading to higher residual cosolvent levels. Thus, from the standpoint of reducing residual TBA levels in the lyophiles, it does not seem necessary to promote drug crystallization from solvents with lower TBA levels.

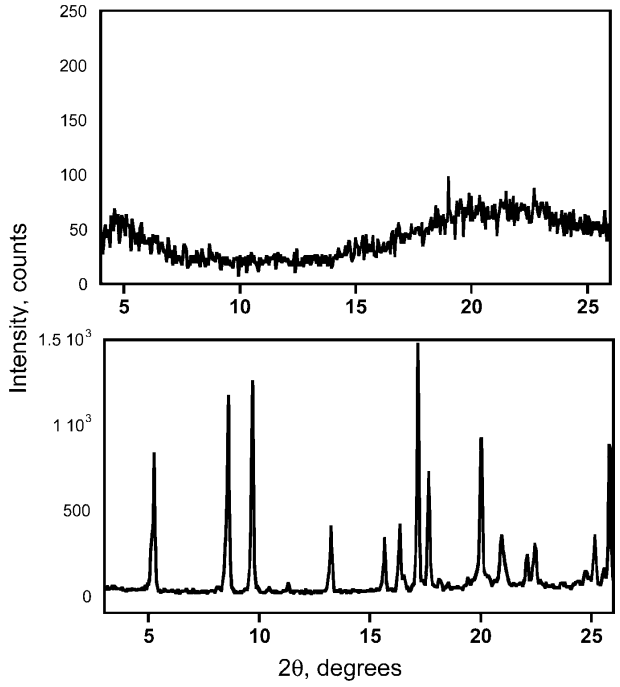

Fig. 6. Powder x-ray diffraction pattern of lyophiles obtained from aqueous (top) and TBA (10% w/w)-water cosolvent solutions (bottom). The initial concentration of **I** (20% w/w) was and the frozen solutions were annealed for 4 h (details in "Materials and Methods" section). Crystalline lyophiles were obtained in presence of other cosolvents as well.

At this stage, it may be helpful to review some of the approaches used to crystallize drugs to evaluate the above results. In commercial freeze-drying, freezing is typically conducted at low cooling rates of ~0.5°C/min. Solutes crystallize more readily at lower rates, since supercooling is minimized and ice crystallization tends to be complete. The annealing step involves an isothermal hold at a temperature above the glass transition of the freeze-concentrate. It is the reduced viscosity and the consequent increase in molecular mobility that accelerates crystallization of the freeze-concentrate. During this hold, devitrification of ice is also possible, resulting in maximal concentration of the solute. Care must be taken to ensure that the annealing temperature is below the onset of ice melting.

Use of crystallizing excipients and IPA as a cosolvent were successful approaches to induce solute crystallization (12,28). It was proposed that the presence of IPA in the freeze-concentrate (18) lowered the Tg', thereby enhancing the mobility during the annealing step (mobility being proportional to temperature above Tg', i.e., T – Tg'). Extending

Table I. Crystallinity of Cephalothin Sodium Lyophiles Obtained from Different Solvent Systems

Solvent system	Drug (% w/w)	Cosolvent (% w/w)	Thermal treatment[a]	% crystallinity[b]
Water	20	None	+	3.6 (0.8)
TBA-water	20	10	+	76.5 (7.3)
	20	10	–	3.1 (0.6)
	10	10	+	4.6 (0.8)
	20	5	+	78.8 (3.8)
Ethanol-water	20	10	+	64.9 (4.3)
	20	10	–	7.9 (0.4)
	10	10	+	14 (1.7)
	20	5	+	63.3 (7.4)
IPA-water	20	10	+	80.6 (4.4)
	20	10	–	6.2 (1.1)
	10	10	+	26.5 (5)
	20	5	+	71.6 (3.5)

[a] −15°C for 4 h; '–' no annealing.

[b] Crystalline standard: commercial sample; amorphous standard: lyophilized sample from aqueous solution without thermal treatment; SD in parenthesis; n = 3.

this reasoning to the four solvent systems of choice (aqueous being the control), in light of the low freezing temperatures and presence of cosolvents in the freeze concentrate, a significant increase in mobility would be expected only in the presence of ethanol and IPA. The small fraction of TBA remaining in the freeze-concentrate as a result of supercooling or crystallization inhibition is rapidly removed on warming past Tg' as seen in Fig. 2. Though TBA did not lower the Tg' of the freeze-concentrate, it was effective in inducing solute crystallization. Thus, lowering of Tg' cannot entirely elucidate drug crystallization. It is also significant that, in cosolvent systems, a lower initial drug concentration of 10% w/w did not yield a crystalline lyophile, which confirms that the degree of supersaturation plays a critical role in facilitating crystallization. Suzuki *et al.* have crystallized **I** from supersaturated aqueous solutions with a short annealing step. (Supersaturated aqueous solutions were prepared by dispersing cephalothin sodium in water at >5°C, followed immediately by cooling to <5°C. The drug was rapidly dissolved to obtain a 30% w/w solution.) They report the following optimal conditions for crystallization during freeze-drying: 25–28% w/w **I**, <0.5°C/min cooling rate and thermal treatment at −4°C (29). Annealing of a 22% w/w aqueous drug solution for ~24 h also resulted in solute crystallization (17). With cosolvent systems however, a thermal treatment step of 4 h produced substantially crystalline lyophiles. Because crystallization is believed to occur due to lower solubility of the drug in the organic solvent rich freeze-concentrated solution, the crystallinity values obtained in this study from solutions containing 5% w/w solvent are comparable to those from higher concentrations of cosolvent.

Lyophile Characteristics and Residual Cosolvent Levels

It is common knowledge that crystalline and amorphous matrices require different processing approaches. For crystalline solutes, while the eutectic melting temperature dictates the highest primary drying temperature without product meltback, in amorphous matrices, primary drying is usually conducted below the Tg' of the freeze-concentrate to avoid collapse. In most of the lyophiles freeze-dried from IPA, ethanol or water, the product had collapsed, separated from the sides of the vial or was stuck to the top (Fig. 7B, top). Products from TBA however, were distinct from all the other systems. The porous lyophiles had a smooth elegant appearance and were friable.

The microscopic appearance of the lyophiles revealed very different morphologies. A flaky solid was formed with an aqueous solvent (Fig. 7, bottom). When IPA or ethanol was present in the formulation, large plate-like crystals were formed. Note that the magnification in Figs. 7B and 7C is ten times that of the micrograph shown in Fig. 7A. Thus, cosolvents in general, reduced the particle size and produced smaller crystals. Comparison of Fig. 7B with 7C reveals that TBA produced very fine needle-shaped crystals. This could be related to the effect of TBA on the morphology of ice to yield needle-shaped crystals (14). Likewise, Ni *et al.* also obtained needle-shaped crystals of an anti-tumor drug when freeze-dried from TBA (9).

Drying from unfrozen solvents takes place by evaporation from the liquid residue rather than sublimation of a solid. Thus, this could often result in bubbling of the liquid and frothing at the surface. It is not uncommon for turbulent deposition of solid to occur at the surface of the product (4), eventually yielding an unacceptable product (Fig. 7B, top). It has also been proposed that the solute may be trapped in liquid droplets of cosolvent with consequent deposition as spherical residues when the liquid evaporates during drying. We attribute the spherical structures seen on crystals of **I** from some ethanol or IPA samples to this drying mechanism (Fig. 7B, bottom).

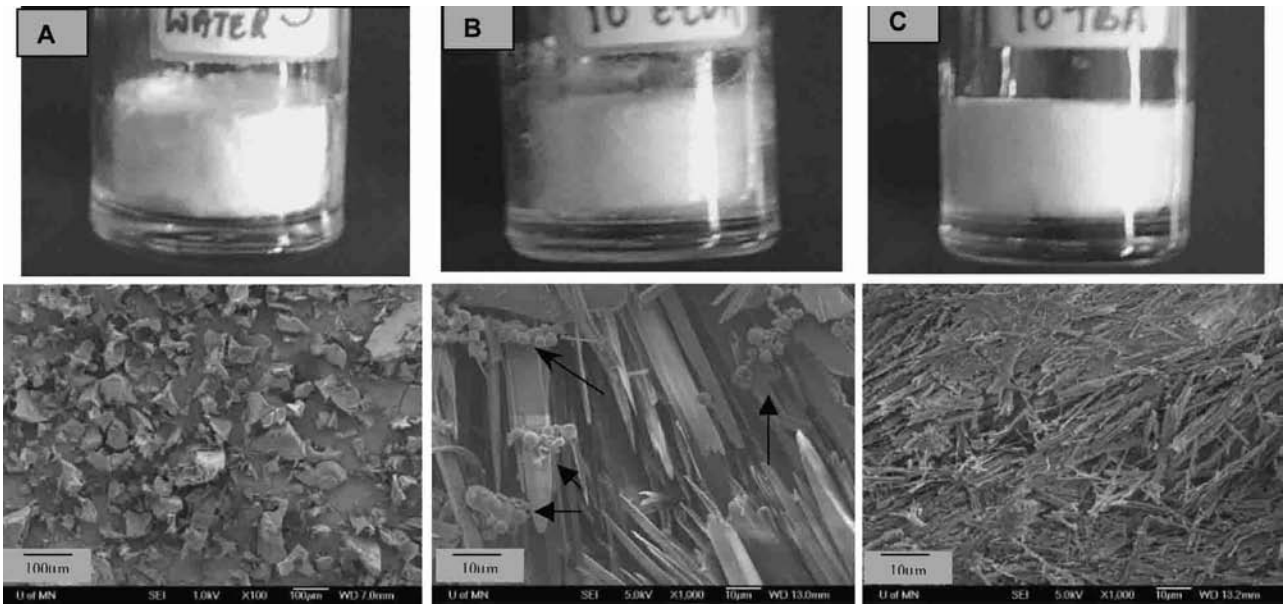

Fig. 7. Macroscopic (top) and microscopic (bottom) characteristics of lyophiles from aqueous and cosolvent systems. (A) water, (B) ethanol, (C) TBA. Cakes obtained from TBA did not show shrinkage. Crystals were small and needle-shaped, while those from IPA or ethanol were much larger and platelike. Spherical structures seen on the surface are marked (B). Note that the magnification in (B) and (C) is ten times that in (A). The prelyo solution contained 20% w/w **I** and 10% w/w cosolvent (if present). The frozen solutions were annealed.

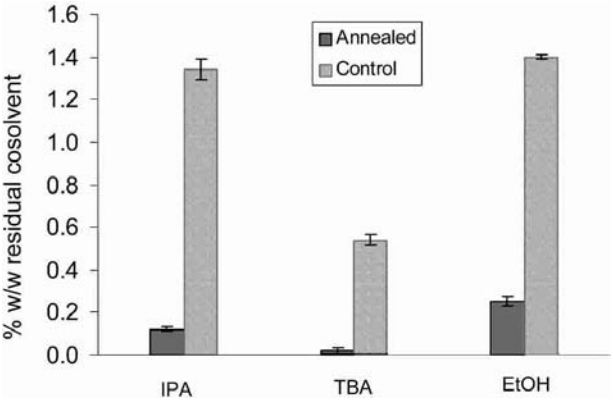

Fig. 8. Residual cosolvent levels (mean ± SD; n = 3) in lyophiles.

Residual solvent levels in annealed and control samples have been compared (Fig. 8). It is evident that the residual solvent levels in annealed samples were very low (<0.2% w/w). TBA levels were lower in both groups. This is a direct consequence of its ability to crystallize during freezing and subsequently sublime. Retention of volatiles has been a subject of interest and relevance in the food science industry. Considerable investigation has been directed toward gaining an understanding of the mechanisms involved in retention of food aromas. Flink and Karel postulate that freeze-concentration of carbohydrate solutions results in formation of "microregions" containing highly concentrated solutions of carbohydrates and volatiles (30). The carbohydrate orients during freezing and drying to surround the volatiles and prevents their escape. Wittaya-areekul and Nail have critically evaluated the impact of formulation and processing variables on residual TBA levels from excipient matrices (21). While sucrose and lactose retained higher levels of TBA when the latter failed to crystallize, a crystalline matrix such as glycine retains negligible amount of TBA, independent of initial TBA concentration or freezing rate. Correlation of residual cosolvent levels with the physical state of **I** lyophiles are in reasonably good agreement with their observations (Fig. 9). Cosolvent concentrations are inversely related to the crystallinity of the lyophiles. Amorphous samples had up to 2.1% w/w cosolvent, while TBA levels in crystalline lyophiles were negligible.

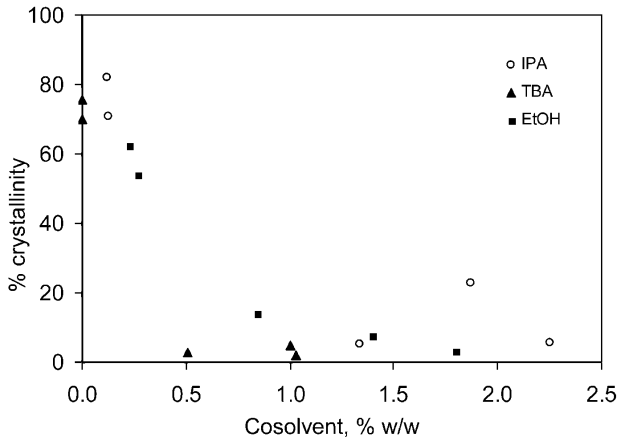

Fig. 9. Correlation of residual cosolvent levels with the degree of crystallinity of **I**. Table I contains all the lyophile compositions.

Ethanol and IPA fall in Class III according to the ICH guidelines established for acceptable solvents (31). Such solvents exhibit low toxicity potential in man. Teagarden and Baker have systematically compiled acute toxicity data for several solvents (5). Although TBA is not listed in the ICH regulatory guidelines, based on its toxicity data (5), it is likely that TBA would also be considered a Class III solvent.

In earlier investigations, two approaches were taken in an effort to obtain crystalline cefazolin sodium at the end of the freeze-drying cycle: isopropyl alcohol was used as a cosolvent and crystallizing excipients were added to the prelyo solution (12,18,28). Compared with cefazolin sodium, the compound under investigation, cephalothin sodium, appears to be more resistant to crystallization. There was no solute crystallization in frozen solutions even after long periods of annealing (23). In addition to successfully promoting solute crystallization, we have focused on lyophile attributes such as crystal morphology and residual cosolvent content. By including TBA as a cosolvent, the crystallization of cephalothin sodium was greatly facilitated. It is likely that the utility of TBA could be extended to facilitate crystallization of other solutes. Preliminary experiments revealed crystallization of cefazolin sodium and lactose, when lyophilized from TBA-water systems. However, when compounds crystallize as hydrates during freezing or annealing (as was the case with cefazolin sodium), dehydration during drying may result in an amorphous end product. Such a crystalline hydrate → amorphous anhydrate transition will negate the utility of the cosolvent. Moreover, because of the strong propensity of amorphous phases to sorb water and organic solvents, the lyophile may have unacceptably high levels of cosolvent.

CONCLUSIONS

Crystalline lyophiles were obtained from a TBA-water solvent system. A short annealing protocol as well as relatively high initial concentration of **I** were determined to be necessary for inducing crystallization in presence of organic solvents. The use of TBA as cosolvent resulted in lyophiles with desirable macroscopic and microscopic characteristics and negligible residual cosolvent levels. Elegant and friable lyophiles were obtained wherein the drug crystals had a fine needle-shaped morphology. The use of TBA for increasing crystallinity may be extended to other amorphous drug candidates.

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