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RESEARCH PAPER

Salt formation during freeze-drying - an approach to enhance indomethacin dissolution

Seema Thakral [1] • Raj Suryanarayanan [1]

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ABSTRACT

Purpose (i) Prepare a freeze-dried injectable indomethacin (IMC) dosage form. (ii) Convert IMC to its tris salt during freeze-drying so as to facilitate rapid dissolution (reconstitution). (iii) Modulate salt crystallinity by annealing the frozen solution.

Methods Aqueous IMC solutions buffered with tris were freeze dried, with or without annealing the frozen solutions. The lyophiles were characterized by X-ray diffractometry, differential scanning calorimetry and infra-red spectroscopy and also subjected to water sorption and dissolution studies.

Results Based on IR spectroscopy, the final lyophile was confirmed to contain the IMC tris salt. In the absence of annealing, the lyophile was X-ray amorphous with a glass transition temperature of 19°C. Annealing the frozen solutions caused a substantial increase in lyophile crystallinity. Interestingly, both the amorphous and partially crystalline lyophiles dissolved "instantaneously" and completely in the dissolution medium. In contrast, the crystalline IMC as well as its physical mixture with tris exhibited much slower dissolution with ~ 50% drug dissolved in 30 min.

Conclusion *In situ* IMC tris salt formation resulted in an elegant lyophile with a very short reconstitution time. Tris served two roles – as a buffer in the prelyophilization solution and as the counterion for the salt in the final lyophile. This approach for solubility enhancement could be extended to other acidic drugs wherein salt formation was observed during freeze-drying.

KEY WORDS annealing · freeze-drying · indomethacin · IR spectroscopy · salt · tris

ABBREVIATIONS

DE Dissolution efficiency
IMC Indomethacin

INTRODUCTION

Active pharmaceutical ingredients intended for parenteral administration may lack adequate stability to be formulated as ready-to-use solutions. In such instances, they can be lyophilized (freeze-dried) and, right before use, this powder can be reconstituted into a solution and administered. This dry state stabilization technique is used for thermolabile small drug molecules as well as proteins and other biological products (1). In addition to parenteral solution formulations, freeze-drying is also used to manufacture orally disintegrating tablets and to improve the long-term storage stability of liposomes and colloidal nanoparticles (2, 3).

The first step in freeze-drying process is cooling, resulting in ice crystallization and consequently, freeze concentration of the solutes. The freeze concentration can have several other effects of pharmaceutical interest and significance. The increase, often dramatic, in the ionic strength of the freeze concentrated solution can facilitate protein denaturation or osmotic dehydration of microorganisms (4). Rates of chemical reactions, specifically second-order reactions, can be accelerated as was observed in the oxidation of ascorbic acid (5). Freeze concentration may also lead to unintended chemical reactions in freeze-dried products. Lyophilization of bortezomib boronic acid with mannitol caused the formation of a mannitol boronic ester. The reconstituted solution consisted of the mannitol ester in equilibrium with its hydrolysis product, the monomeric boronic acid (6). Freeze-drying

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✉ Raj Suryanarayanan
surya001@umn.edu

[1] Department of Pharmaceutics, College of Pharmacy, University of Minnesota, 308 Harvard Street SE, Minneapolis, Minnesota 55455, USA

of a binary solution of 1,3-diamino-2-hydroxypropane and citric acid resulted in an amorphous salt in the lyophile (7). Likewise, freeze-drying of N-acetyl–L-cysteine solution with magnesium carbonate resulted in the formation of the magnesium salt of N-acetyl–L-cysteine (8).

The process of freeze-drying can also render a solute amorphous, an attribute desired for rapid reconstitution. However, amorphous compounds can be less stable than their crystalline counterparts (9). The other features of amorphous phases, for example the tendency to sorb water and the stochastic nature of crystallization, may also make them undesirable. Solute crystallization from the freeze concentrate can be facilitated by annealing. During annealing, the frozen solution is held isothermally at a temperature above T_g', the glass transition temperature of the freeze concentrate. In order to accelerate solute crystallization and facilitate complete crystallization, the annealing temperature should be between the T_g' and T_{eu} (crystalline solute – water eutectic temperature) of the system (10). Annealing can alter the crystal habit of ice and yield hexagonal stellar dendrites (11). In addition, there can be Ostwald ripening, a phenomenon whereby large ice crystals grow at the expense of the small crystals. The consequent coarsening of the frozen matrix has been shown to reduce vial-to-vial heterogeneity in drying rate (12, 13). On the other hand, annealing below T_g' can promote crystallization of the unfrozen water associated with the amorphous phase. The amorphous phase deplasticization is manifested by a measurable increase in the glass transition temperature (4, 14). The consequent supersaturation facilitates solute nucleation (15).

Indomethacin (IMC; free acid), the drug of interest, has low aqueous solubility (5 μg/mL at 25°C; γ-IMC) and is susceptible to hydrolysis (16, 17). The carboxylic acid group (pKa = 4.5) in the molecule enables salt formation and IMC is commercially available, both as a sodium and as a meglumine salt (18, 19). The sodium salt is used parenterally in the treatment of patent ductus arteriosus. In spite of its commercial availability, there are numerous challenges and unresolved issues with this injectable IMC formulation (20, 21). Therefore, our first object was to use freeze-drying to prepare an IMC salt with an organic compound, tromethamine (tris) (22). Since the salt was intended for parenteral administration, very rapid reconstitution (dissolution) was a prerequisite for effective product use. The second object was to use annealing to control the physical form of the salt and thereby modulate its physicochemical properties. Tris, owing to its inherent ability to act as a buffer at the proposed pH, was expected to play a dual role. The protonation of the primary amino group in the tris enables it to act as a cation (the anion being the carboxylate group of IMC molecule). The final solution pH of 7.7 being within one unit of the pKa of tris (8.0), the need for a separate buffer is obviated.

MATERIALS AND METHODS

Materials

IMC (γ-form) was purchased from CSPC Ouyi Pharmaceutical Co. Ltd. (Shijiazhuang, China), and tris(hydroxymethyl)amino methane (tris; purity > 99%) from Sigma-Aldrich. These chemicals were used without further purification. A pH meter (Mettler Toledo), calibrated with standard buffer solutions (Oakton standard buffers; pH 2.00, 4.01, 7.00, and 10.00 at 24 ± 1°C; certified by NIST) was used.

Lyophilization

An IMC slurry was prepared to which tris was added and the slurry sonicated for ~ 60 s to facilitate dissolution (final concentrations of IMC and tris were 0.10 and 0.15 M respectively; pH 7.7). The solution was filtered (0.2 μm PTFE filter, Acrodisc CR), filled into 10 mL glass vials (2 mL fill volume), covered with rubber stopper (20 mm, 2 LEG LYO, GRY, BBTYL, Wheaton) and loaded into the lyophilizer. Lyophilization was carried out in a bench top freeze-dryer (VirTis AdVantage, Gardiner, NY). The shelf was cooled to -45°C at 0.25°C/min and held for 2 h. Primary drying was conducted at -30°C (65 ± 25 mTorr) for 20 h. During secondary drying, the shelf was progressively heated to −10, 0, +10 and + 25°C and held at each temperature for 6.6 h. At the end of the cycle, the vials were stoppered and stored in a desiccator containing anhydrous calcium sulfate at -20°C.

Selected batches were annealed by modifying the thermal treatment steps of the freeze-drying cycle. Thus after holding the shelves at -45°C for 2 h, the temperature was ramped to -10°C and held for 12 h. The shelf was cooled back to -45°C, held for 2 h, before initiating primary drying at -30°C. All the other process parameters were the same. Annealing was also carried out under two other conditions: -5°C for 6 h or -15°C for 32 h.

IR spectroscopy

Spectra (Vertex 70, Bruker, Ettlingen, Germany; equipped with a globar mid-IR source) were obtained using an attenuated total reflectance (ATR) accessory (single reflection germanium crystal) and a DLaTGS detector. The resolution was 4 cm^{-1}, and 32 scans were acquired in the range of 4000–400 cm^{-1}. The peak positions were determined using OPUS software peak picking function.

X-ray diffractometry

A powder X-ray diffractometer (D8 ADVANCE; Bruker AXS, Madison, WI) equipped with a Si strip one-dimensional detector (LynxEye) was used.

Samples were exposed to Cu Kα radiation (40 kV × 40 mA) over an angular range of 5 - 35° 2θ with a step size of 0.02° and a dwell time of 0.5 s.

Differential scanning calorimetry

A differential scanning calorimeter (Q2000, TA Instruments, New Castle, DE) equipped with a refrigerated cooling accessory was used. Dry nitrogen gas was purged at 50 mL/min. For thermal analysis of prelyo solution, ~20 μL of solution was weighed in an aluminum pan, sealed hermetically, cooled from RT to -50°C at 1°C/min, held for 30 min and heated to RT at 10°C/min. Selected systems were annealed wherein the solutions were cooled from RT to -50°C at 1°C/min, held for 30 min, heated to the annealing temperature (this ranged between -20 and -5°C; 5°C/min), annealed for the desired time (this ranged between 1 and 5 h), cooled back to -50°C at 5°C/min and heated to RT at 10°C/min. In case of lyophiles, the powder was filled into the aluminum pan at RT (in a glove box under nitrogen purge; RH ≤ 5%), sealed non-hermetically, and heated from RT to 180°C, at 10°C/min. The more specific experimental details are provided in the appropriate figure legends.

Scanning electron microscopy

The powder samples were placed on aluminum stubs using a double-sided carbon tape, coated with platinum (50 Å) and subjected to scanning electron microscopy (Jeol 6500 F, Hitachi, Japan).

Dissolution

A United States Pharmacopeia Type 2 dissolution testing apparatus (Varian 705 DS, Varian, Palo Alto, CA) was used. The dissolution medium (pH 7.2 phosphate buffer; 900 mL) was maintained at 37 ± 0.5°C and the paddle speed was 100 rpm. Fifty milligram of IMC (or the equivalent amount in case of the lyophile) was added to the dissolution medium, samples withdrawn periodically, filtered (0.45 μm membrane filter), and assayed spectrophotometrically (Cary Bio 100 spectrophotometer, Varian, Walnut Creek, CA; 320 nm).

Water vapor sorption

About 5 mg of sample was placed in the quartz sample pan of an automated vapor sorption balance (DVS-1000, Surface Measurement Systems, London, UK), maintained at 25°C. It was dried under dry nitrogen purge (flow rate 200 mL/min) to constant weight. The relative humidity (RH) was first increased to 5%, and then progressively increased to 95%, at increments of 10%. Equilibrium was assumed to have been achieved if the mass change was < 0.005% over 30 min. The microbalance was calibrated periodically using a 100 mg standard weight.

RESULTS AND DISCUSSION

The results are divided into four sections. (i) Characterization of the solution prepared for lyophlization (hereafter referred to as 'prelyo solution'). (ii) The freeze-drying cycle including the annealing conditions. (iii) Characterization of the final lyophiles. (iv) Generation of the phase and state diagram of the IMC tris-water system.

Characterization of prelyo solution

Our efforts to prepare IMC solution in tris buffer, at equimolar concentrations of drug and buffer (0.1 M), were unsuccessful. The drug was not completely soluble and the buffer concentration had to be increased to 0.15 M to completely dissolve the drug. The final solution pH was 7.7. At this pH, IMC (pKa 4.5) will be almost completely ionized, while ~65% of tris (pKa 8.0) will be ionized (concentration of protonated tris ~ 0.0975 M). Thus the molar concentrations of the ionized acid (drug) and base (buffer) were approximately the same at pH 7.7.

In all our subsequent experiments, a drug to buffer molar ratio of (1:1.5) was maintained. However, for the sake of simplicity, only the IMC concentration will be mentioned.

Thermal analysis

The DSC heating curve of the frozen prelyo solution exhibited a baseline shift at ~ −33°C, attributed to the glass transition of the freeze concentrate (T_g'). This was followed by an endotherm at ~ −0.4°C due to ice melting (Fig. 1). In many systems of pharmaceutical interest, at temperatures > T_g', solute crystallization had been observed (23, 24). There was no evidence of solute crystallization in our system (note the absence of exotherm even in the inset; no crystallization observed even at slow heating rates (Fig S1: Supplementary material). However, since the solute concentration was low (0.1 M IMC), instrumental sensitivity can become a limiting factor.

The IMC concentration was increased up to 2.0 M in the prelyo solution. Even at the highest solute concentration, there was no evidence of crystallization. Interestingly, the T_g' appeared to be independent of the solute concentration (Fig. S2: Supplementary material). Thus, irrespective of the initial solute concentration, amorphous freeze-concentrate of constant composition was obtained. In systems where the solutes remain amorphous, the primary drying temperature is dictated by the T_g'. When a frozen solution is primary dried at temperatures above the collapse temperature (T_c), the product

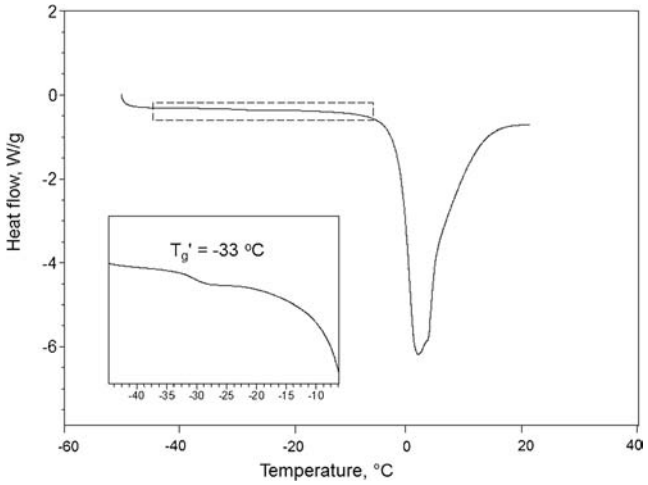

Fig. 1 DSC heating curve of frozen prelyo solution (0.1 M). The prelyo solution was initially cooled from RT to −50°C at 1°C/min, held for 30 min, and heated to 25°C at 10°C/min. A select region has been expanded to enable the visualization of the glass transition of the freeze-concentrate (T_g'). The midpoint of T_g' is reported. Only the heating curve is shown.

may lose macroscopic structure and collapse during freeze-drying (25, 26). Since the T_c is usually a few degrees higher than the T_g', primary drying was conducted at -30°C.

Though there was an "excess" of tris (0.05 M) in the IMC tris prelyo solution, there was no evidence of T_g' of tris (~ −55°C). There was also no indication of tris crystallization. On the other hand, DSC of tris (0.05 M) solution revealed crystallization at ~ −24°C and eutectic at ~ −5°C (Fig. S3: Supplementary material). These results strongly suggest that the excess tris did not phase separate and the freeze concentrate consisted of IMC, tris and unfrozen water.

From the DSC of the frozen solutions, there was no indication of solute crystallization. Furthermore, when the solution was freeze-dried, the lyophile was X-ray amorphous (details presented later). It is well known that, chemically, amorphous compounds are less stable than their crystalline counterparts (9). It was therefore of interest to obtain a crystalline lyophile. In an effort to crystallize the solute, the frozen solutions were annealed. The annealing temperature ranged from −20 to −5°C. At each of these temperatures, the annealing time ranged from 1 to 5 h (Fig. S4; supplementary material). If solute crystallization was brought about by annealing the frozen solution, the T_g' would no longer be observed (assuming complete solute crystallization). At -10°C, we increased the annealing time to 12 h. DSC no longer revealed a T_g' suggesting substantial if not complete solute crystallization. A lower temperature of -15°C, required a longer annealing time of 32 h for solute crystallization (data not shown).

Freeze-drying cycle

The prelyo solutions were yellow in color (Fig. 2a). These solutions were annealed either at -10°C for 12 h or at -15°C for 32 h. The unannealed solution served as the control and the yellow color was retained, both in the frozen solution (Fig. 2b) and in the final lyophile (Fig. 3a). On the other hand, when the frozen solution was annealed, either at -10 or at -15°C, it was no longer yellow (Fig. 2c) and yielded a white lyophile. Thus the unannealed system yielded a yellow cake while annealing resulted in a desirable white lyophile (Fig. 3).

Lyophile characterization

IR spectroscopy

The IR spectra of amorphous IMC, tris and the lyophile are overlaid in Fig. 4. In IMC, let us first consider the asymmetric carbonyl stretch due to cyclic acid dimer (1708 cm^{-1}) and the shoulder at 1735 cm^{-1}, attributed to non-hydrogen bonded carbonyl stretch. Both these peaks were absent in the IR spectrum of lyophile presumably due to the "loss" of the carboxylic acid proton. The new peak at 1568 cm^{-1} in the lyophile can be attributed to asymmetric carboxylate stretch. Thus there is indication of ionization of IMC carboxylic acid (and the consequent formation of carboxylate ion) in the lyophile. The shoulder at 1390 cm^{-1}, attributed to symmetrical carboxylate stretch, provided supporting evidence of loss of proton from IMC. The formation of salt of primary amine was evident from the broad shoulder starting from 1568 and extending to ~1525 cm^{-1}. There was also evidence of asymmetric bend at 1596 cm^{-1} (though not very distinct). Asymmetric -NH_3^+ bend is known to result in absorption bands between 1625 and 1560 cm^{-1} while the bands of symmetric -NH_3^+ bend are observed over 1550–1505 cm^{-1} (27). This confirms the transfer of proton from the carboxylic acid group of IMC to the primary amine functionality in tris, resulting in the formation of the tris salt of IMC. Detailed characterization of IMC tris salt is presented in the supplementary material (Figs. S5-S7). Several other acidic drugs including ketorolac, dinoprost, lodaxamide and fosfomycin are available as tris salts (28–30). The formation of the sodium, lysine and arginine salts of IMC during freeze-drying is also known (31, 32).

As pointed out earlier, the molar ratio of IMC to tris was 1.0:1.5. This "excess" tris is expected to be retained in the lyophile. Its presence was confirmed by the amine doublet at 3348 cm^{-1} (asymmetric) and 3289 cm^{-1} (symmetric), and the -NH_2 bend at 1587 cm^{-1}. As expected, these signals were weak. While the final lyophile contains IMC tris salt and tris, for the sake of simplicity, we refer to the lyophile only as the salt, while recognizing the presence of tris.

Fig. 2 Photograph of: (**a**) prelyo solution at RT, immediately after loading into the freeze-dryer. (**b**) Frozen solution - before annealing. (**c**) Frozen solution - after annealing.

X-Ray diffractometry

Figure 5 shows the XRD patterns of IMC, tris and the lyophiles. In the absence of the annealing step, the lyophile was X-ray amorphous. Annealing at -5°C for 6 h yielded an X-ray amorphous yellow lyophile (data not shown). A possible explanation is that ice melting could have started and thereby inhibited solute crystallization. Annealing the frozen solution at a lower temperature, either at -10°C for 12 h or at -15°C for 32 h, yielded a partially crystalline powder. These two annealing conditions yielded powders with comparable degrees of crystallinity.

The crystalline lyophiles obtained after annealing were characterized by lines with d-spacings of 10.1 (8.74 °*2θ*), 6.79 (13.1 °*2θ*), 5.04 (17.5 °*2θ*), 4.15 (21.4 °*2θ*) and 3.48 Å (25.5 °*2θ*). These peaks could be attributed neither to tris [tris is characterized by lines with d-spacings of 6.29 (14.1 °*2θ*), 4.9 (18.1 °*2θ*), 4.43 (20.0 °*2θ*), 3.97 (22.4 °*2θ*) and 2.64 Å(33.9 °*2θ*) Å] nor to the γ-polymorph of IMC [characteristic d-spacings of 8.69 (10.2 °*2θ*), 5.21 (17.0 °*2θ*), 4.51 (19.6 °*2θ*), 4.07 (21.8 °*2θ*) and 3.04 Å (29.4 °*2θ*)] . The α- polymorph of IMC is characterized by intense lines at 18.22 (4.8 °*2θ*), 7.79 (11.3 °*2θ*), 6.13 (14.4 °*2θ*), 3.71 (23.9 °*2θ*) and 3.63 Å (24.5 °*2θ*) and none of them were observed in the lyophile. At room temperature, only one polymorphic form of tris has been observed though it is known to undergo reversible solid-solid transformation at ~ 136°C (33). Thus the XRD results support our earlier conclusion, based on IR spectroscopy, of IMC tris salt formation. Several peaks (for example with d-spacings of 6.29 (14.1 °*2θ*), 4.90 (18.1 °*2θ*), 4.43 (20.0 °*2θ*) and 3.97 Å (22.4 °*2θ*) attributable to tris were also observed in the powder pattern of the lyophile. This could be explained by the crystallization of the "excess" tris (IMC to tris molar ratio of 1.0:1.5) in the prelyophilization solution.

Thermal analysis

The lyophile obtained without annealing the frozen solution exhibited a single glass transition at ~19°C (Fig. 6). Interestingly, the T_g of the lyophile was lower than that of both IMC (~44°C) and IMC sodium (T_g ~ 121°C). The high T_g of the sodium salt was ascribed to the strong ionic interaction between sodium and IMC ions resulting in a pronounced reduction in free volume (32). For IMC-meglumine binary melt, a single T_g of 50°C has been reported (34). Various studies have highlighted the importance of counterions in influencing the glass transition temperature of amorphous salts (35–37).

The lyophile obtained after annealing the frozen solution revealed several thermal events - an endotherm at ~ 87°C, an exotherm at ~ 100°C followed by an endotherm at ~ 141°C. TGA revealed a weight loss of 1.0% between RT and 90°C. The lyophile water content was determined to be 1.1% w/w by Karl Fischer titrimetry. Hence the first endotherm is attributed to loss of water from the lyophile. In order to characterize the other thermal events observed at elevated temperatures, detailed TGA, IR and variable-temperature XRD studies were conducted. The results suggested a combination of

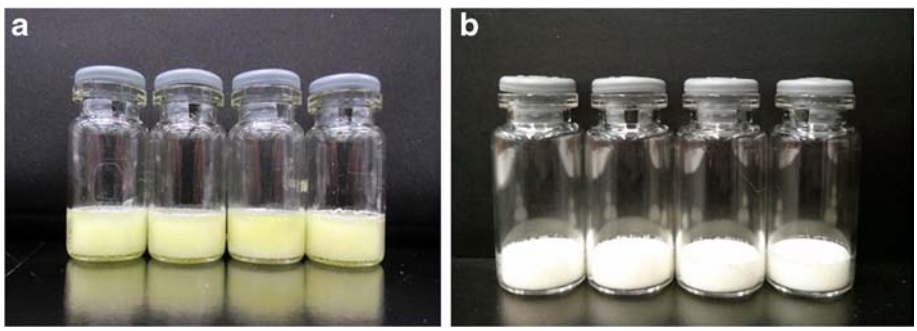

Fig. 3 Effect of annealing on the appearance of the final product. Lyophile obtained (**a**) without annealing, and (**b**) after annealing the frozen prelyo solution.

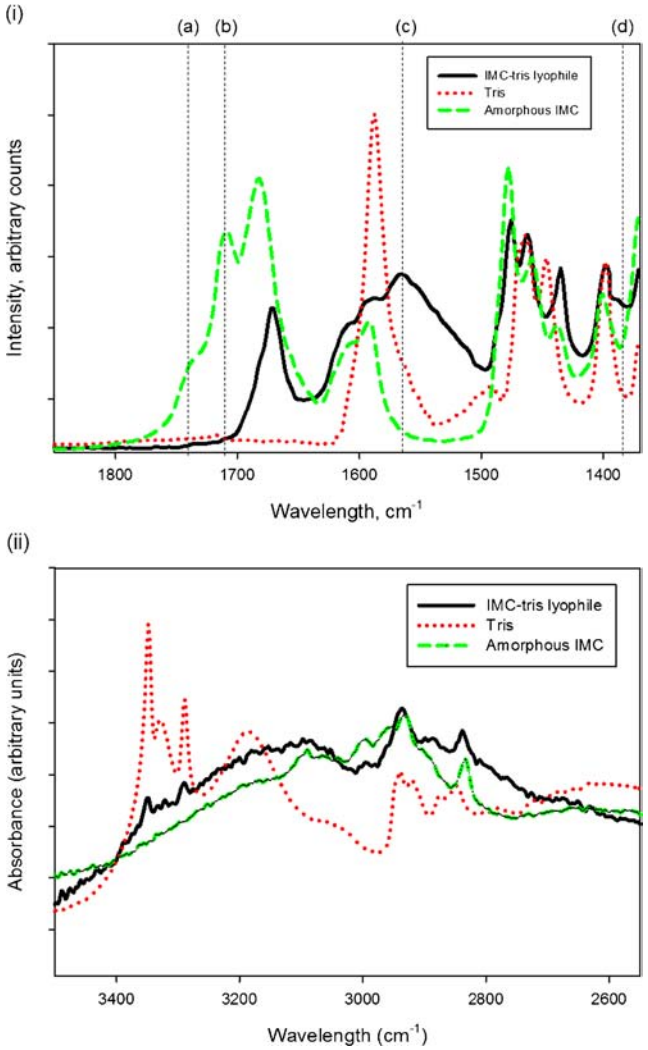

Fig. 4 FT-IR spectra of IMC (rendered amorphous by melt quenching), tris ("as is") and IMC tris lyophiles (obtained after annealing). (**i**) 1850 – 1370 cm^{-1}. *Vertical broken lines*: (**a**) carbonyl stretch due to non-hydrogen bonded acid (observed in the free acid), (**b**) asymmetric carbonyl stretch due to cyclic acid dimer (observed in the free acid), (**c**) asymmetric stretch due to carboxylate anion (observed in the salt), and (**d**) symmetric stretch due to carboxylate anion (observed in the salt, as a weak shoulder). (**ii**) 3500 – 2550 cm^{-1}.

physical and chemical transformations and the results are included in the supplementary section (Section 3, Figs. S8-S10).

Scanning electron microscopy

Exposure of the unannealed (yellow colored) lyophile to ambient conditions (25°C/~40% RH) led to its instantaneous collapse (not shown). On the other hand, annealing of the frozen solution yielded a "stable" lyophile which did not collapse even when exposed to ambient conditions for several hours. The lyophiles were also subjected to scanning electron microscopy (Fig. 7). In the absence of annealing, a highly fluffy porous powder was obtained (Fig. 7a). Exposure to ambient conditions (for 10 min) led to collapse and fusion,

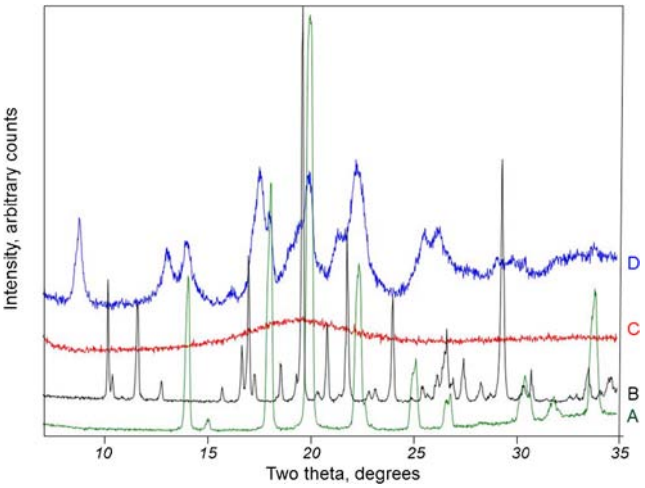

Fig. 5 The XRD patterns of (*A*) freeze-dried tris, (*B*) crystalline IMC (γ-polymorph; "as is"), (*C*) lyophile without annealing step, and (*D*) lyophile obtained after annealing the prelyo solution (-10°C for 12 h).

disappearance of pores and formation of a continuous seamless bulk structure (Fig. 7c). Annealing led to a radical change in the microstructure and yielded plate-like particles with pronounced edges suggesting a crystalline lyophile (Fig. 7b). On exposure to air, the plate-like macrostructure was largely retained (Fig. 7d).

Dissolution profile

The dissolution profiles of crystalline IMC and the lyophiles are presented in Fig. 8. A physical mixture of crystalline IMC and tris (1:1.5 molar ratio) also served as a control. Crystalline IMC dissolved gradually with cumulative dissolution of 77 and 88% of the drug in 60 and 90 min respectively. This translated to IMC solution concentrations of ~ 38 and

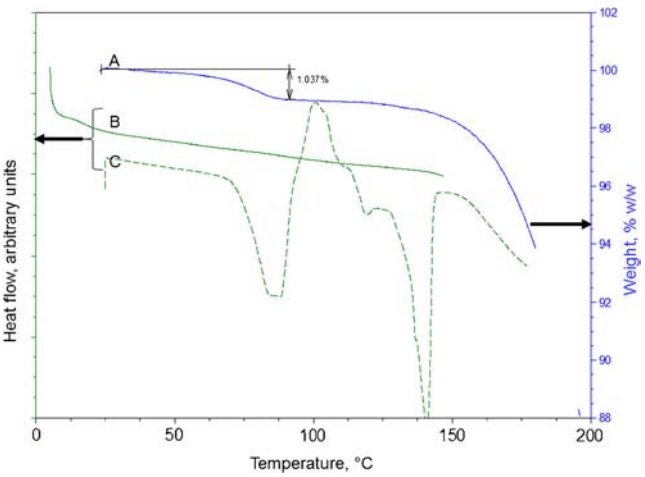

Fig. 6 Thermal analysis of lyophiles. (*A*). Thermogravimetric analysis (TGA) of lyophile obtained after annealing the frozen solution at -10°C for 12 h (right y-axis). (*C*). The corresponding DSC heating curve (left y-axis). (*B*). DSC heating curve of lyophile obtained without annealing (left y-axis).

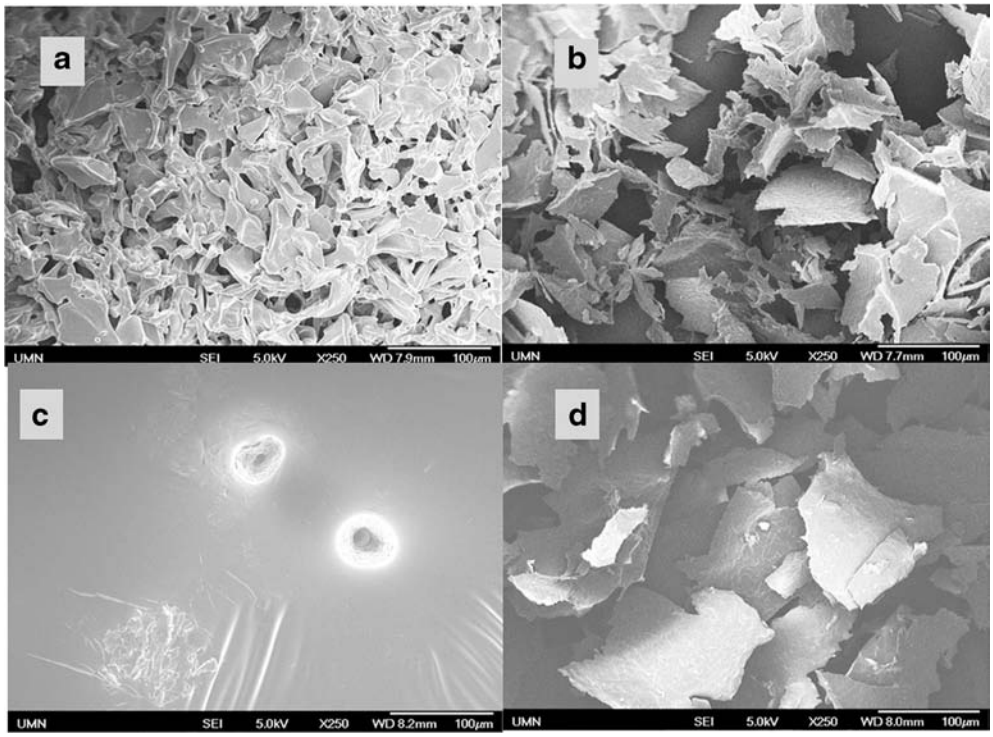

Fig. 7 Scanning electron microscopy of freshly prepared lyophiles (**a**) without annealing, and (**b**) after annealing the frozen solution. (**c**) (unannealed) and (**d**) (annealed) are the corresponding lyophiles exposed to ambient conditions for 10 min.

44 µg/mL respectively, in broad agreement with the reported of IMC solubility of ~ 40 µg/mL (32°C) at pH 7 (38). The dissolution of IMC did not cause any measurable change in the medium pH. The presence of tris (IMC tris physical mixture) appeared to slightly accelerate the dissolution rate of IMC. Remarkably, the lyophile dissolution profiles revealed complete and "instantaneous" dissolution. Importantly, the dissolved drug remained in solution with no evidence of precipitation. The pronounced acceleration in dissolution can be explained by the increase in solubility brought about by the salt formation (IMC tris). It is interesting that the dissolution behavior exhibited by both the crystalline (annealing the frozen solution at -10°C for 12 h) and the amorphous (no annealing) lyophiles were virtually identical. Under the experimental conditions, the physical form of the salt (crystalline or amorphous) did not influence the dissolution behavior.

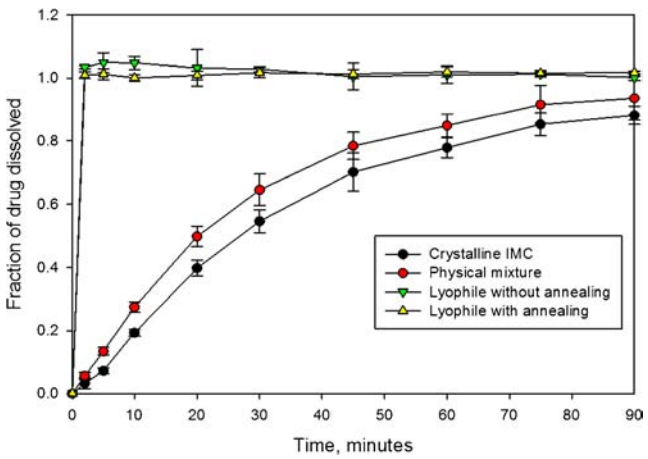

Fig. 8 Dissolution profile of (i) crystalline IMC, (ii) physical mixture of crystalline IMC and tris, (iii) lyophile obtained without annealing, and (iv) lyophiles obtained with annealing the frozen solution ($n = 3$).

Dissolution efficiency (DE) is a model independent parameter used to compare dissolution profiles of multiple formulations (39). DE_t, the dissolution efficiency at time t, is calculated using equation:

$$DE_t = \frac{\int_0^t y(t).dt}{y_{100}.\ t} \quad (1)$$

where *y(t)* denotes percent drug dissolved up to any time *t* while the denominator denotes the area of rectangle (plot of percent drug dissolved *versus* time) representing 100% dissolution in the same time. The dissolution efficiency of the different systems were evaluated at 2, 10 and 90 min and the results are presented in Table I. The dissolution medium was first sampled at 2 min and, even in this short time period, IMC dissolution from the lyophile was complete. More importantly, during the entire dissolution study (90 min), there was no evidence of IMC recrystallization. The much slower dissolution of crystalline IMC and the IMC tris physical mixture is reflected by their low DE values.

Water vapor sorption

The commercially obtained IMC and tris exhibited negligible water uptake over the entire RH range (Fig. 9; experiments at

Table I Dissolution Efficiency (Eq. 1) of IMC Lyophilized with Tris at Different Stages of the Dissolution Run

Sample	DE_2	DE_{10}	DE_{90}
IMC Crystalline	1.6 ± 0.9[a]	8.5 ± 1.2	60.2 ± 2.8
IMC tris physical mixture	2.8 ± 0.6	13.6 ± 1.3	67.8 ± 6.0
Lyophile obtained without annealing	51.2 ± 1.3	93.7 ± 1.1	100.8 ± 1.7
Lyophile obtained with annealing	50.9 ± 1.3	91.2 ± 1.1	100.2 ± 0.9

The data at 2 (DE_2), 10 (DE_{10}) and 90 min (DE_{90}) are provided (Fig. 8). The crystalline IMC and the IMC tris physical mixture served as controls.

[a] Mean ± SD; $n = 3$.

25°C). However, at 95% RH, tris deliquesced. The lyophiles, obtained with or without the annealing step, readily sorbed water and at RH ≥ 50%, the difference in their water sorption was readily discernible (Fig. 9; inset). Annealing, by causing partial crystallization, substantially decreased the water sorption propensity.

Partial phase and state diagram

As mentioned earlier, the ice melting temperatures of solutions with different solute concentrations (up to 2 M IMC), were obtained by DSC (Fig. 10; triangles) and yielded the partial phase diagram. The details of the generation of the diagram are given in the figure legend.

Earlier, the water sorption studies had revealed that the lyophiles (unannealed) were amorphous and had a strong tendency to sorb water. Their glass transition temperatures as a function of water content are plotted as circles (solute weight fraction ≥ 0.66). The glass transition temperatures as a function of solute weight fraction were calculated using the Fox equation (Eq. 2):

$$\frac{1}{T_g} = \frac{w_1}{T_{g_1}} + \frac{w_2}{T_{g_2}}. \quad (2)$$

Here w_i and T_{g_i} are respectively the weight fraction and the glass transition temperature of component i and T_g is the glass transition temperature of the mixture. The calculated and the experimental T_g values were in very good agreement particularly at high solute weight fractions. However, as the solute weight fraction decreased (≤0.80), the observed T_g values were higher than the calculated values. This can be explained by the crystallization of a fraction of the unfrozen water. The ice melting observed in the DSC at these compositions (solute weight fractions of 0.80 and 0.66; blue square) provided direct evidence of ice formation.

DSC of the prelyo solutions, irrespective of the initial solute concentration (solute weight fraction ≤ 0.49), consistently revealed a T_g' of ≈ −33°C, reflecting a freeze-concentrate of constant composition. From these results, the T_g' (glass transition temperature of the freeze-concentrate) and W_g' (composition of the freeze-concentrate expressed as solute weight fraction) were estimated to be −33°C and 0.81 respectively.

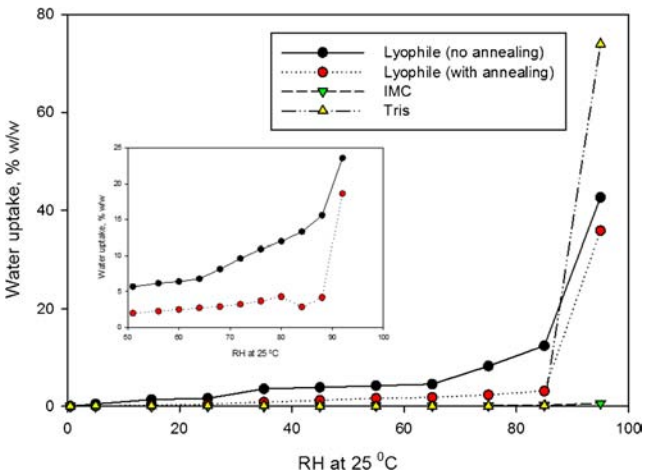

Fig. 9 Water sorption profiles of crystalline IMC ("as is"; γ-form), tris ("as is"), and final lyophiles. The effect of annealing on the water sorption behavior of the lyophile is evident from the inset.

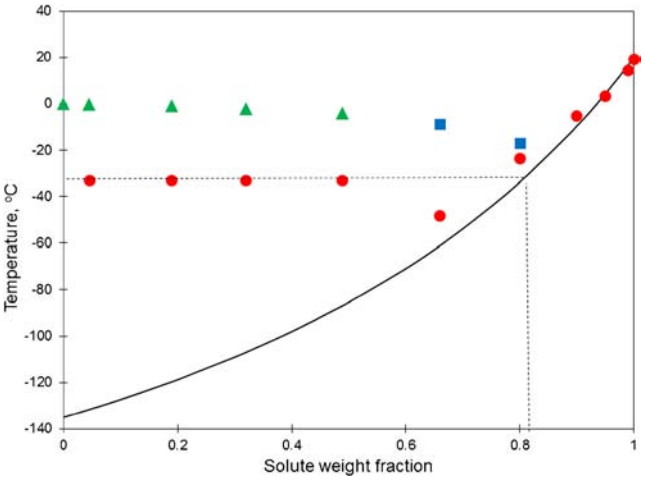

Fig. 10 Partial phase and state diagram of IMC tris salt – water* system. For determining the ice melting temperature of the frozen prelyo solution (*green triangle*), IMC tris salt solutions of different concentrations were cooled to −50°C, held for 30 min and heated to 25°C at 5°C/min. These DSC profiles also yielded T_g' of the freeze-concentrate (up to a solute weight fraction of 0.49). The rest of the T_g' values (water weight fraction ≤ 0.34) were obtained by allowing lyophiles to sorb a predetermined amount of water following which they were subjected to DSC**. These T_g' values are represented by the *red circles*. Ice melting was also observed at solute weight fractions of 0.80 and 0.66 (*blue squares*). The solid line represents the calculated glass transition temperatures using the Fox equation (Eq. 2). The T_g' of the freeze-concentrate appeared to be independent of the prelyo solution composition (*horizontal discontinuous line*) enabling the estimation of W_g' (composition of the freeze-concentrate). *The molar ratio of IMC to tris was 1:1.5. ** The sample was cooled to −60°C at 15°C/min, held for 30 min and then heated to 50°C at 5°C/min.

Significance

In the pharmaceutical community, so far, freeze-drying has found application as the dry state stabilization technique of choice for thermolabile pharmaceuticals. Herein, we have demonstrated *in situ* salt formation during freeze-drying leading to desirable changes in the physicochemical properties of the drug. Since the buffering agent served as the cation (the only excipient in the system), there was no need to add an additional excipient which would yield the counter-ion for salt formation.

At the pH of the prelyophilization solution (pH 7.7; 3 units > pKa), the drug was almost completely ionized leading to the salt formation. The tris salt dissolved much faster than the parent compound (IMC; free acid). The high dissolution rate was also manifested in the near instantaneous lyophile reconstitution in water. In addition, the processing conditions enabled us to control the physical form of the final lyophile. While annealing the frozen solution yielded a crystalline lyophile, an amorphous product was obtained otherwise. Amorphous systems, in light of their higher free energy than their crystalline counterparts, will exhibit more rapid dissolution. Thus, if a crystalline solute dissolves slowly leading to unacceptably long reconstitution times, the amorphous form may provide the required dissolution advantage. However, we observed very rapid dissolution of both the partially crystalline and amorphous lyophiles with no discernible difference between the two.

The *in situ* salt formation is not unique to indomethacin. Solutions of ibuprofen and aceclofenac buffered in tris yielded the respective crystalline tris salts (40). These types of chemical interactions in a multicomponent formulation during the process of freeze-drying cannot be ruled out and warrant consideration during preformulation and formulation studies.

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