Thermal Analysis of the Tertiary Butyl Alcohol-Water System and Its Implications on Freeze-Drying

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Utilizing differential scanning calorimetry (DSC) for tertiary butyl alcohol (TBA) solutions a phase diagram was constructed for the TBA-water system. By utilizing thermal treatment during the DSC measurements the metastable states were eliminated. The phase diagram fit that for a congruently melting compound in which compound formation occurred with a maximum at 70% TBA representing pure TBA hydrate. Two eutectics occurred at 20% (Eutectic A) and 90% (Eutectic B). A freeze-drying microscope revealed that TBA altered the crystal habit of ice. A concentration of 3% TBA was required before large needle-shaped ice crystals became evident. The addition of 10% TBA to the system resulted in even finer needle-shaped ice crystals. At the eutectic compositions (20% and 90% TBA), the frozen eutectic mixture could not be resolved with the microscope because eutectic crystals are very small. The 70% TBA solution, which corresponds to the melting of pure TBA hydrate, formed very large hydrate crystals. The rate of sublimation of the TBA and water molecules was found to be concentration dependent. At concentrations below 20% TBA (water rich portion of the phase diagram) water molecules sublimed faster while at concentrations above 20% TBA (TBA rich portion of the phase diagram) TBA molecules sublimed faster. At the eutectic A composition, both TBA and water molecules sublimed at the same rate. This may be because all of the TBA molecules are strongly associated with each other in the form of a clathrate hydrate.

KEY WORDS: lyophilization; tertiary butyl alcohol; freeze-drying microscope; phase diagram; crystal habit.

INTRODUCTION

Drug solutions may contain various concentrations of organic solvents which are often added to reduce the degradation rate of the drug in water or to increase solubility (1,2). The presence of these organic solvents has been shown to affect the freezing characteristics of the solution intended for freeze-drying, a phenomenon which subsequently affects the rate of drying and the physical appearance of the freeze-dried product (2).

The solvent of interest in this study was tertiary butyl alcohol (TBA), an organic substance which has been shown to increase the efficiency of the freeze-drying process (3). Because of the growing interest in using TBA in freeze-drying, both as a mass-transfer accelerator and solubilizing agent, a thorough thermal analysis was undertaken using Differential Scanning Calorimetry (DSC). The thermal analysis was done to identify and eliminate the metastable states so that a phase diagram which would be applicable to freeze-drying could be constructed. The metastable states do not occur consistently as has been implied in previous studies of the TBA-water system (4,5). The thermal transitions in the various regions of the phase diagram were then assessed using a freeze-drying microscope. Finally, the sublimation rates in these regions were determined using a freeze-dryer equipped with a sample thief.

MATERIALS AND METHODS

Materials

Tertiary butyl alcohol (Fisher Scientific, Fairlawn, NJ); freeze-drying microscope (specially made freeze-drying stage with a Zeiss microscope, Eli Lilly & Company); freeze-dryer (Hull Model 10FXS12C, Hartboro, PA) equipped with a sample thief; toluene (Fisher Scientific, Fairlawn, NJ); *n*-butanol (Fisher Scientific, Fairlawn, NJ); gas chromatograph (Varian 3500, Walnut Creek, CA).

Thermal Analysis of the TBA-Water System

Distilled TBA and filtered deionized water were used in this study. A differential scanning calorimeter (DSC) (Perkin-Elmer TAS-7) series equipped with a 5000 series computer) was used for the analysis. Solutions of various concentrations of TBA and water were prepared by weight. About 10–15 mg of the sample solution were pipetted into an aluminum pan and the pan was capped with a hand press to prevent evaporation of the solution. The pan was placed in the sample compartment and an empty pan was similarly capped and placed in the reference compartment. The solution was frozen at a rate of 5°C/min to −40°C and held at this temperature for 5 minutes to allow for complete solidification. The solution was heated at a rate of 2.5°C/min to 25°C.

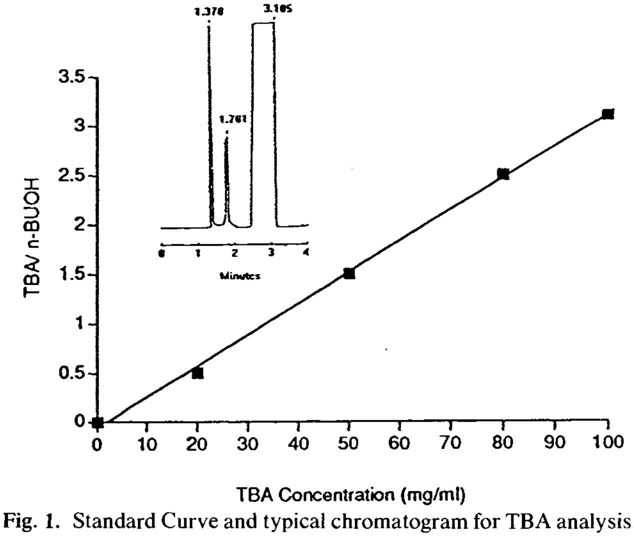

Fig. 1. Standard Curve and typical chromatogram for TBA analysis. Retention times are TBA, 1.378 min; *n*-butanol, 1.781 min; toluene, 3.185 min.

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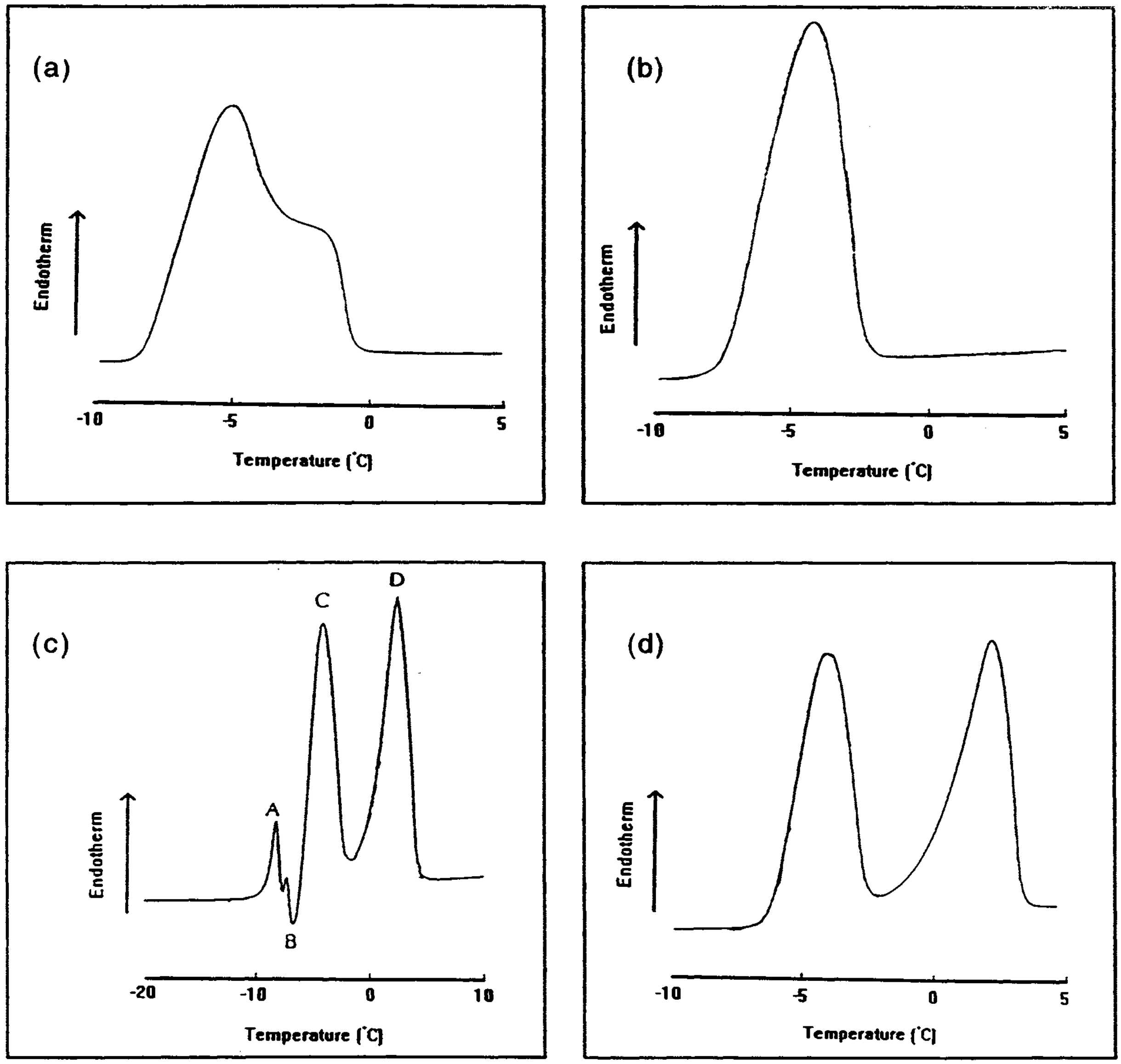


Fig. 2. DSC warming thermograms for TBA-water mixtures, a) 15% w/w TBA; b) 20% TBA; c) 50% TBA solution, Endotherm A: melting of metastable eutectic; Exotherm B: recrystallization of metastable eutectic to from stable form; Endotherm C: melting of eutectic, Endotherm D: melting TBA hydrate; d) thermal treatment of 50% solution at −7°C to eliminate metastable states.

Triplicate scans were performed. The temperature at which a thermal event occurred for a given concentration was plotted against the TBA concentration to construct the phase diagram. Metastable thermal events were identified and excluded in the phase diagram. For example, at high concentrations (>20% w/w) of TBA, two metastable thermal events occurred. The first event was endothermic and the second exothermic. When the frozen solution was warmed to −7°C, held at this temperature for 20 minutes, then cooled to −25°C and rewarmed, these events were no longer present.

Crystal Structure of TBA-Water Mixtures

A freeze-drying microscope was created by interfacing a high vacuum cold stage with an optical microscope (specially made freeze-drying stage with a Zeiss microscope, Eli Lilly & Company). This system was used to examine the crystal structure of frozen TBA-water mixtures. A drop of the sample solution was placed on a 24 × 24 mm coverslip and spread evenly by covering it with an 18 × 18 mm coverslip. Silicone stopcock grease was applied to the bottom of the coverslip and the temperature controlled plate. A temperature probe was also glued to the coverslip with silicone grease. The temperature of the cold stage was controlled by passing cold nitrogen gas through the channels in the plate. The sample solution was cooled to −40°C and a polaroid camera was used to photograph the changes.

Freeze-Drying of Aqueous TBA Solutions

Aqueous solutions containing 10%, 20%, 44%, and 80% by weight of TBA were prepared and filtered. Preweighed 10 ml Type I borosilicate vials (West Co.) were filled with 5 ml of the sample solution, reweighed and transferred to the freeze-dryer. The solutions were cooled to −45°C and held at this temperature for 2 hours to allow for complete solidification. Sublimation of the solutions was undertaken at a shelf temperature of 0°C and a chamber pressure of 100 mTorr. Samples were removed via a sample thief at various stages of drying for TBA and water analysis.

TBA and Water Analysis

Sample Preparation. The vials removed during the cycle were weighed and the weight loss was measured. Five ml of toluene was used to extract the TBA by shaking the vials for 30 seconds and then allowing 10 minutes for complete separation of aqueous and organic phases. 0.08 ml of *n*-butanol was added to the toluene extract as an internal standard. One μl of the organic phase was injected into a gas chromatograph with an FID detector (Column: Rtx-1 methylsilicone, 20 m × 0.25 mm, Detector temp: 280°C, Injector temp: 200°C, Column temp: 35°C, Plot speed: 1.5 cm/min). Figure 1 shows the standard curve and a typical chromatogram with the TBA peak at 1.378 minutes.

Standard Curves. Aqueous solutions of TBA were prepared at various concentrations.

TBA was extracted with five ml of toluene. 0.08 ml of *n*-butanol were added to the extracts and the solutions analyzed as above. The ratio of the peak area of TBA to peak area of *n*-butanol was plotted against TBA concentration. This standard curve was used to quantitate the amount of TBA in the sample. Subtraction of the mass of TBA from the total weight of the solution gave the amount of water remaining.

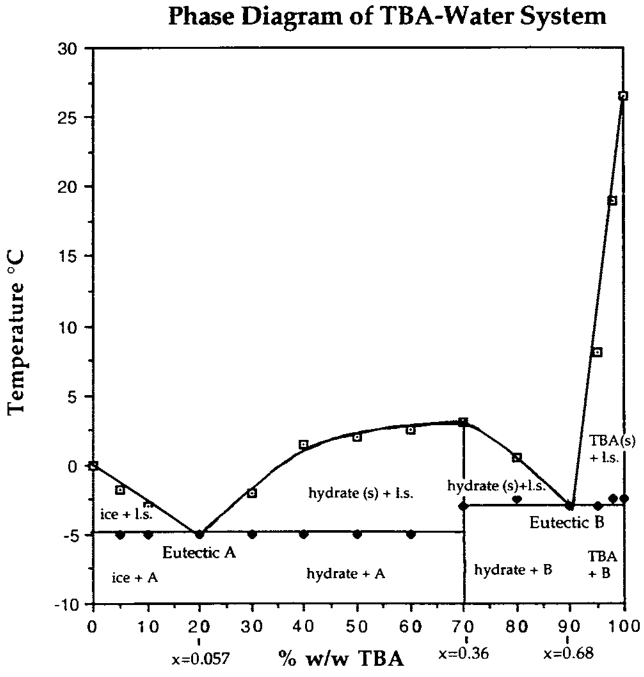
RESULTS AND DISCUSSION

Thermal Analysis

A total of 14 selected concentrations of TBA in water were run in triplicate. Figure 2 (a–d) shows the DSC warming thermograms of the TBA-water mixtures. Figure 2a is the warming thermogram of a 15% TBA solution. The first endotherm was due to the melting of the eutectic and the second endotherm to the melting of ice. The thermogram for the 20% TBA solution (Fig. 2b) had only one endotherm due to the melting of the eutectic. The DSC thermogram of solutions having concentrations above 20% such as that in Figure 2c had three endotherms and one exotherm. At 50% TBA the first endotherm (A) occurred at −10°C. The second event (B), an exotherm, was followed immediately by the melting of the eutectic endotherm (C). The third endotherm (D) was due to the melting of the TBA hydrate. These events were also observed by Kamat *et al.*, in his study of the TBA-water system (3). The first endotherm (A) was believed to be a metastable thermal event because it was followed by a sudden recrystallization (B). To prove that (A) was metastable, the frozen sample was heated to −7.0°C and kept at this temperature for 20 minutes. The sample was cooled to −25°C and warmed a second time. The thermogram of the second heating of the 50% TBA solution had only two peaks indicating that (A) was metastable.

The DSC data was used to construct the phase diagram for the TBA-water system as represented in Figure 3. This type of phase diagram is indicative of compound formation. A common occurrence is for Water and TBA to form a third compound, a TBA hydrate (4,5). The complex phase diagram can be described as consisting of two simple eutectic phase diagrams placed side by side. The left side represents the simple eutectic phase diagram for water-TBA hydrate and the right side that for the TBA hydrate-TBA. The maximum in the phase diagram at 70% TBA corresponds to the melting of the pure TBA hydrate (4,5). The TBA-water system has two eutectic compositions, one at 20% TBA (eutectic A) and the other at 90% TBA (eutectic B). Eutectic A occurs at −5°C for concentrations below 70% TBA and eutectic B occurs at about −3°C for concentrations greater than or equal to 70% TBA. Upon cooling a solution of TBA, solid TBA, ice or solid TBA hydrate will separate out depending on the concentration of the solution. Typically, in our studies only 5–10% TBA was used for the purpose of accelerating freeze-drying cycles (3). This concentration falls in the water rich portion of the phase diagram. During cooling of such a solution, pure ice will form leaving behind an increasingly concentrated TBA solution. As the temperature is continually lowered, more ice will precipitate until a point is reached where both TBA and water will crystallize out as eutectic A. This occurred at 20% TBA for the water-TBA hydrate system.

The phase diagram of the TBA-water system has been published by several investigators (6,7). Ott *et al.*, constructed the phase diagram of the TBA-water system using thermal methods (a platinum resistance thermometer was used to measure the onset of thermal events). They reported that TBA formed a dihydrate which melted at 273.5°K and an



x: TBA concentration on mole fraction basis

Fig. 3. Phase diagram of the TBA-water system plotted on % (w/w) basis. Mole fractions are listed for Eutectic A and B and the pure hydrate.

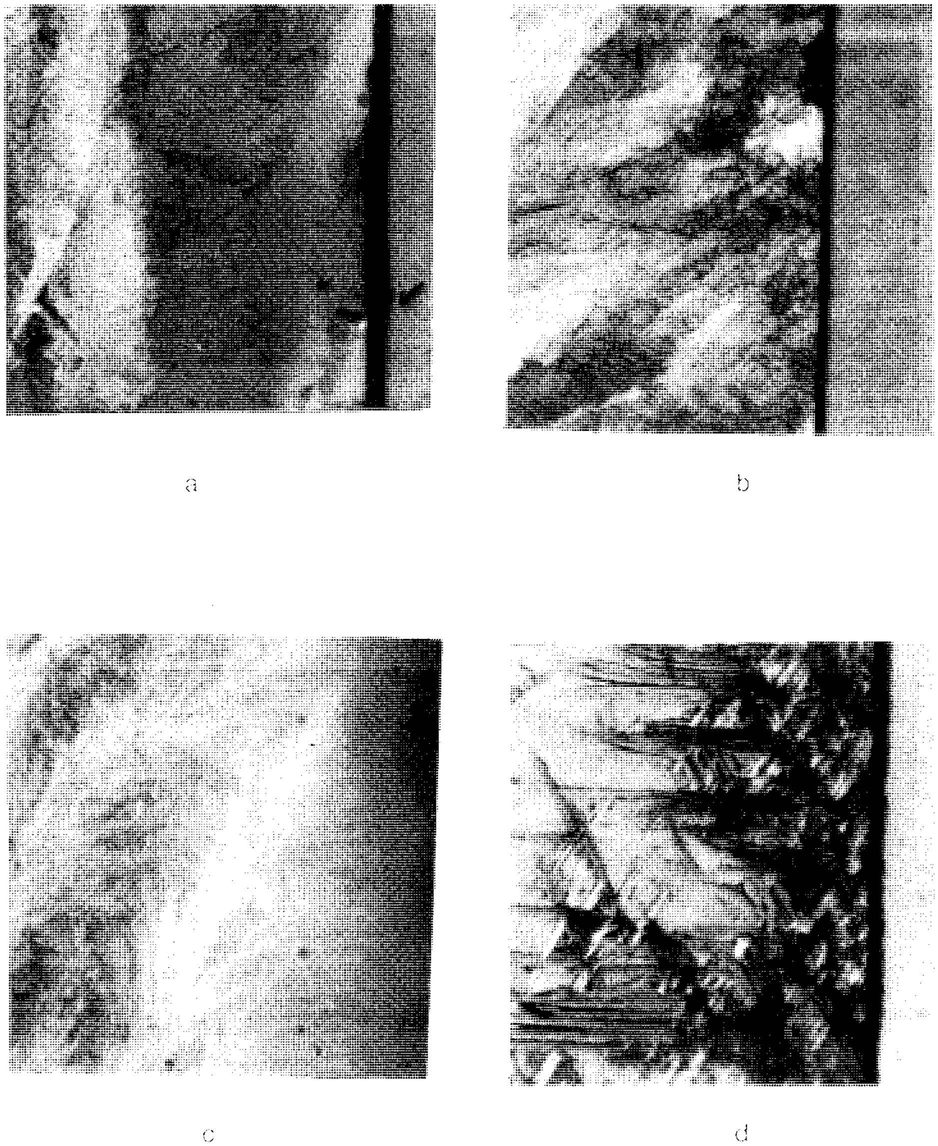


Fig. 4. Polaroid photographs of frozen TBA-water mixtures. a) Frozen deionized water; b) 1% w/w TBA solution; c) 3% TBA; d) 10% TBA.

incongruently melting hexahydrate. The shape of our phase diagram is in general agreement with the one reported by Ott as is the presence of a dihyrate. However, we are not in agreement regarding the formula assigned to the hexahydrate in the water rich side of the phase diagram. Woznyj and Ludemann also constructed the phase diagram of the TBA-water system using differential thermal analysis and compared that with two other authors (5). Again, our phase diagram agrees well with those reported by Woznyj *et al.* The major difference between our work and those reported in literature is the presence of metastable events. Our phase diagram does not include metastable events because we were able to eliminate these using thermal methods. However, Ott *et al.* claim that the metastable phases are uncommonly "stable" and that conversion to the stable phase is difficult. The other major difference between our work and that reported by Ott and Woznyj is the presence of metastable events at concentrations lower than 20% TBA (or 0.060 on a mole fraction basis). At concentrations below 20% metastable events were not always encountered, whereas they report metastable events below 20%. Between 20–70% TBA (0.060–0.36 mole fraction) our results and those reported in the literature agree very well. At the eutectic composition (20% TBA) our phase diagram indicates melting at 268.15°K (or −5°C) while Ott's and Woznyj data shows melting at 265°K and 261°K, respectively. This difference is simply due to the fact that they see metastable events at concentrations less than 20% TBA.

Woznyj *et al.* also reported a strong anomaly of the TBA-water phase diagram around a TBA mole fraction of 0.067 (approximately 22% TBA), which they speculated to be the presence of a hydrate with a TBA/water ratio of approximately 1:14. According to other literature sources, the water-TBA ratio for the hydrate is 17 (8). This agrees quite well with our phase diagram, since 20% TBA (eutectic A) corresponds to about 16 moles of water to 1 mole of TBA.

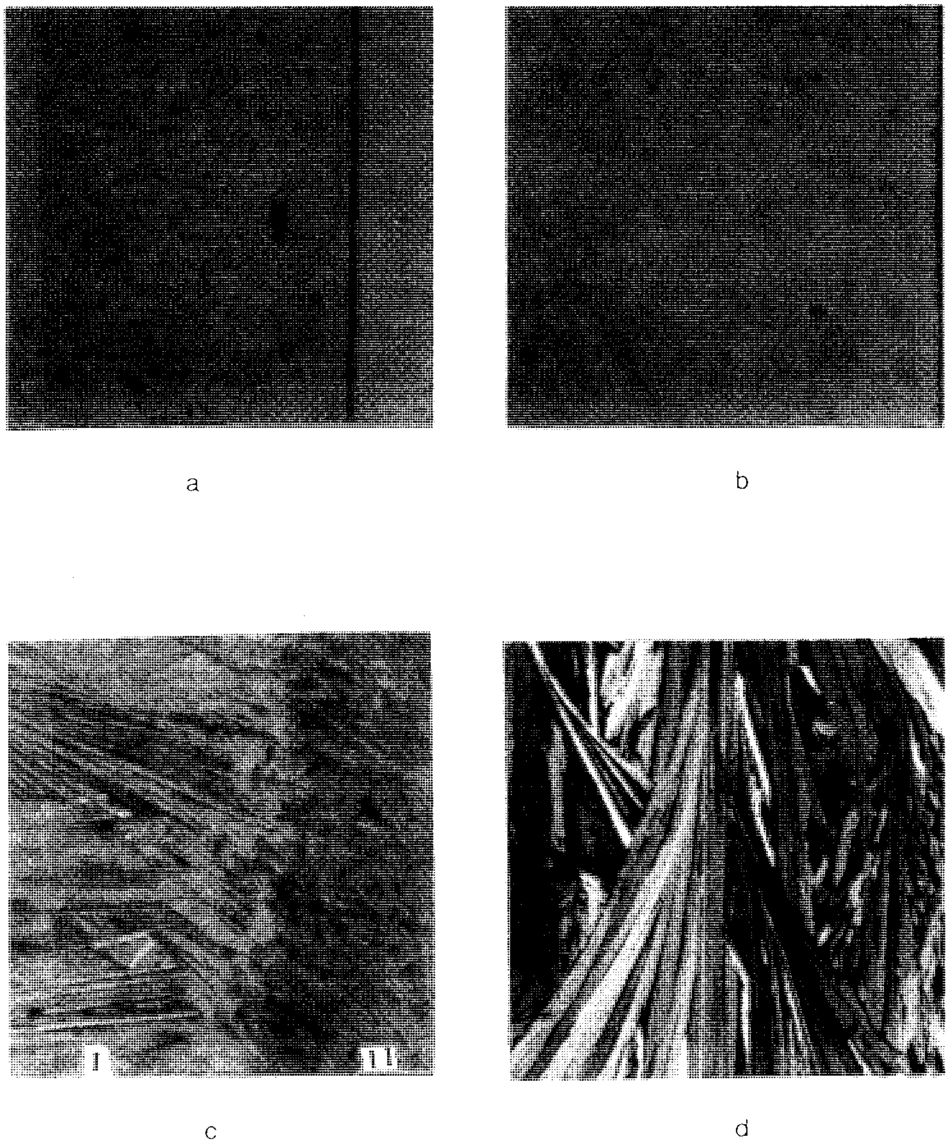


Fig. 5. Polaroid photographs of frozen TBA-water mixtures. a) 20% w/w TBA; b) 90% TBA; c) 50%; d) 70% TBA.

Glew *et al.* examined the effect of ethylene oxide, dioxane, and TBA on the activity coefficient of water (8,9). They found the maximum water stabilization was obtained in solutions containing 4.0, 3.9, and 3.7 mole % ethylene oxide, dioxane and TBA, respectively. Simply stated, at these concentrations the water structure becomes more ordered. It has been postulated that TBA dissolves in two states (10). At low concentrations (<20%) there are enough water molecules to surround the TBA molecule, thus forming stable clathrate hydrates.

Near-infrared spectra have shown that the addition of low concentrations of TBA (<20%) cause the water molecules to become more ice-like (9). However, higher concentrations of these compounds have a destabilizing effect on water structure, because not enough water molecules exist to surround the TBA molecule and hence there is a greater chance for TBA molecules to self-associate. The near-infrared data seem to support Woznyj's claim of an anomaly of the TBA-water system at about 22% TBA.

Structure of the Frozen TBA-Water Mixtures. Figures 4a and 4b are the polaroid photographs of frozen deionized water and a 1% w/w aqueous TBA solution, respectively. The two photographs look similar. The different colors represent the different grain boundaries of ice. Figures 4c and 4d are the frozen aqueous solutions of 3% w/w TBA and 10% w/w TBA, respectively. As the concentration of TBA was increased from 1% to 3% w/w there appeared to be more ordering of the water structure. The frozen solution of 3% TBA had larger more dendritic ice. The solution containing 10% TBA (Fig. 4d) formed needle-shaped ice crystals. When this frozen sample was slowly heated from −45°C to room temperature, small voids became evident at −5°C throughout the sample due to the melting of the eutectic ice (this agreed with DSC studies). At about 0°C the needle-shaped crystals melted, signifying that these were actually ice crystals. The presence of TBA in the solution altered the crystal habit of ice. Some of the factors which influence the crystal habit are supersaturation, rate of cooling, nature of crystallizing solvents, and the presence of cosolvents (11).

Figures 5a and 5b are the photographs of the 20% TBA and 90% TBA solutions, respectively. These solutions represent the two eutectics compositions of the TBA-water system (eutectics A and B). Since eutectic ice crystals are usually small, they could not be resolved under an optical microscope. Solutions containing higher concentrations of TBA were also examined. Figures 5c and 5d are the frozen solutions of 50% and 70% TBA, respectively. The first event during the cooling of the 50% w/w TBA solution was the formation of needle-shaped crystals (Fig. 5c). These have been marked as I. The second event which occurred at the lower temperature was the freezing of the eutectic (marked as II). The needle-shaped crystals formed in the 50% TBA solution are actually TBA-hydrate crystals. The 70% TBA solution, which corresponds to the melting of pure TBA hydrate, formed very large hydrate crystals, as shown in Figure 5d. It should be noted that because the rate of freezing in the freeze-drying microscope is typically faster than that in an actual freeze-dryer it was essential to slow the rate of freezing to permit the type of crystal formation seen in the Figure. Multiple runs at this concentration at faster rates of freezing resulted in smaller hydrate crystals. Similar freezing treatment at the other concentrations did not show the same behavior as seen in 5d for the 70% concentration.

Rate of Sublimation of TBA and Water. As shown in Figure 6, the flux of the solution, i.e., the loss of both water and TBA, increased linearly with increasing concentration of TBA. This was expected since TBA has a higher vapor pressure than water. In order to compare the rate of sublimation of the two molecules, the ratios of moles of TBA remaining to the moles of water remaining were plotted against time in Figure 7 for 10%, 20%, 44%, and 80% TBA solutions. A positive slope was obtained for 10% TBA indicating that water molecules are subliming faster than TBA molecules. This was expected since 10% lies in the water rich portion of the phase diagram. The 44% and 80% solutions have negative slopes, indicating that TBA is subliming faster than the water molecules. From the phase diagram, 44% and 80% TBA fall in the TBA rich portion of the diagram. The 20% TBA solution is of interest since it shows that during the 5 hours of drying at a shelf temperature of 0°C and a pressure of 100 mTorr the molar ratio of the two species remained constant indicating that both molecules sublimed at the same rate. This occurred because the TBA and the water molecules were strongly associated. This association may be in the form of a clathrate hydrate.

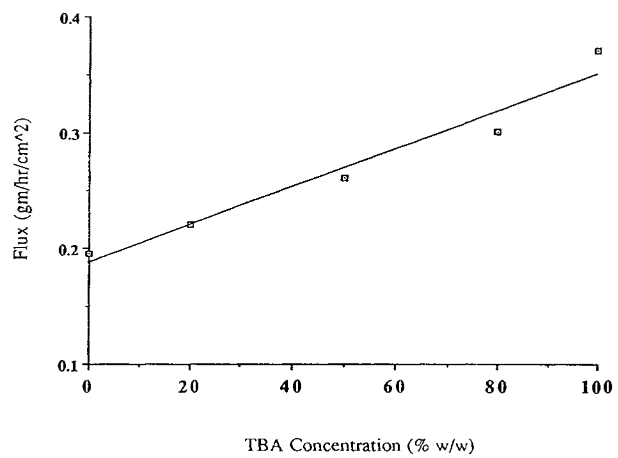


Fig. 6. Flux of TBA/water mixture as function of TBA concentration (freeze-dried at a shelf temperature of 0°C and pressure of 100 Mtorr).

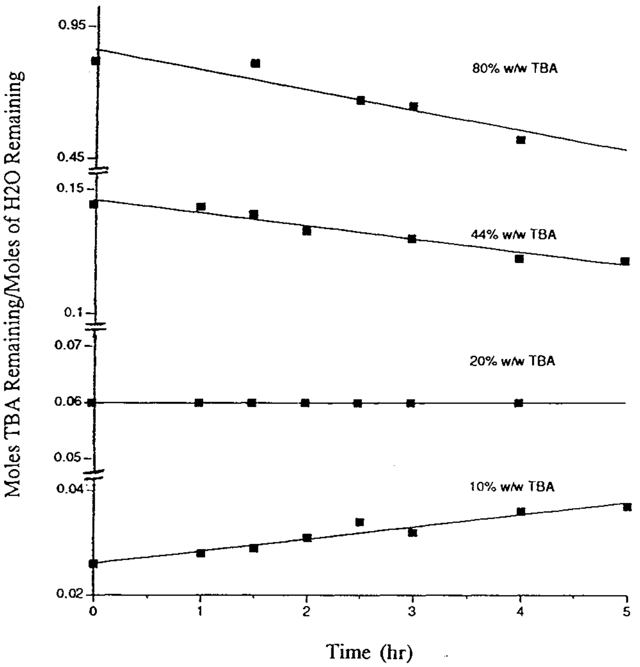


Fig. 7. Ratio of TBA to water as function of time for 10%, 20%, 44%, and 80% TBA solutions.

CONCLUSION

The phase diagram of the TBA-water system is indicative of compound formation. This diagram agrees fairly well with those reported in the literature for the TBA-water system; the major difference being the presence of metastable events in the literature. In this study the metastable states were identified and eliminated by thermal treatment.

The freeze-drying microscope revealed that TBA affects the crystal habit of ice. The addition of 3–19% TBA resulted in the formation of needle-shaped ice crystals, a phenomenon which is important in freeze-drying. Once these large needle-shaped ice crystals sublime, they leave behind a more porous and less resistant dry matrix through which drying can take place.

The rate of sublimation of TBA and water molecules agrees well with the phase diagram TBA molecules sublime faster. During the sublimation of the 20% TBA solution the molar ratio of the two species remained the same. This suggests that both species are associated strongly at this concentration. As previously reported, an anomaly exists at 20% TBA which may indicate the formation of still another hydrate.

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