Eutectic Temperature Determination of Preformulation Systems and Evaluation by Controlled Freeze Drying

RASHMIKANT M. PATEL▲ and ARTHUR HURWITZ

Abstract □ Differential scanning calorimetry was utilized in the preformulation screening of three multicomponent drug systems at subzero temperatures. The results were demonstrated to be directly comparable to those from differential thermal analysis. The effects of formulation additives on differences in melting behavior and eutectic temperature were investigated. The effect of added sodium chloride varied widely, depending on the nature of the other components. Melting point–composition diagrams were constructed, and percent melt values at a given temperature were calculated. Both eutectic temperature and percent melt values were important to the evaluation of various compositions. Finally, controlled lyophilizations were investigated in an attempt to relate percent melt and final product physical appearance.

Keyphrases □ Eutectic temperature—effect of additives, preformulation screening, multicomponent drug systems, differential scanning calorimetry-differential thermal analysis results compared □ Freeze drying—definition of lyophilization, preformulation screening, multicomponent drug systems, differential scanning calorimetry-differential thermal analysis results compared □ Differential scanning calorimetry—preformulation screening, multicomponent drug systems, compared to differential thermal analysis □ Differential thermal analysis—compared to differential scanning calorimetry, preformulation screening, multicomponent drug systems □ Formulations—effect of additives, preformulation screening, multicomponent drug systems, determination of eutectic temperature, lyophilization conditions defined

The importance of eutectic temperature to the design of an optimum lyophilization cycle has been well documented (1–5). Low temperature differential thermal analysis (6–14) and electric resistivity have been applied (1, 4) in detecting eutectic transitions in various systems.

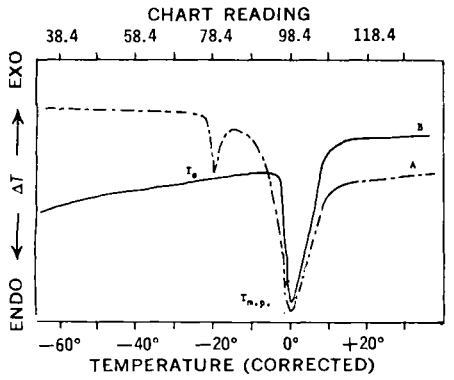

Figure 1—*Differential thermal analysis thermograms. Key: A, water; and B, 2.6% (w/v) aqueous sodium chloride. Heating rate = 10°/min., scale = 20°/in., ΔT = 2°/in., and atmosphere = air.*

Since quantitation is possible, differential scanning calorimetry has been utilized for the determination of purity of organic compounds (15, 16) and freezable water in bread dough (17). The application of low temperature differential scanning calorimetry is not new. However, it has not been used in the detailed analysis of composition variables affecting pharmaceutical preparations to be lyophilized.

This study demonstrates the utility of differential scanning calorimetry and differential thermal analysis in the preformulation evaluation of component levels and lyophilization conditions. The effects of added sodium chloride on eutectic temperature variation for three pharmaceutical systems were determined. From a temperature–composition diagram, the percent melt at a given temperature was calculated. With eutectic temperature and percent melt values, optimization of component addition was possible and definition of lyophilization conditions was achieved.

EXPERIMENTAL[1]

Materials—Three preformulation systems were screened: (A) drug–sodium citrate–lactose–sodium acetate (10:5:75:10), (B) drug–sodium citrate–lactose (10:5:85), and (C) drug–sodium citrate (75:25). Sodium chloride, lactose, and buffer salts were reagent or USP grade.

Calibration of Differential Thermal Analysis—No critical evaluation of reference compounds for subambient temperature is available (18). Various investigators have used chloroform, anisole, and water. For this study, water was selected as a reference material because of convenience, temperature range of intent, and sample character. Glass beads were used as the reference. The samples were cooled to −140° with liquid nitrogen and then scanned on a warming cycle at a heating rate of 10°/min. The melting transitions of ice for nine samples of distilled water were averaged and corresponded to a chart reading of 98.4 ± 1.2. This value was assigned 0°. The endotherm of water is shown in Fig. 1.

For 2.2 and 2.6% aqueous sodium chloride solutions, the eutectic transition (T_e) was observed at the dial reading of 78.0 or −20.4°. A value of −21.2° (corrected) was reported (7) from differential thermal analysis data for 3% aqueous sodium chloride at a heating

[1] The differential scanning calorimeter (Perkin-Elmer DSC-IB) was equipped with a low temperature Dewar flask, a Servo Riter II recorder (Texas Instrument Inc.), and aluminum pans designed to hold liquid samples. The differential thermal analyzer (DuPont model 900) was equipped with a low temperature cell, ceramic sleeves for centering the chromel–alumel thermocouples in macrotubes and charts (Part 900325). A laboratory scale lyophilizer (Hull model 650-4F6) with Honeywell recorder, stainless steel baffle, aluminum metal block [0.61 m. × 0.31 m. × 2.8 cm. (2 ft. × 1 ft. × 1.1 in.)], and other accessories for lyophilization was used.

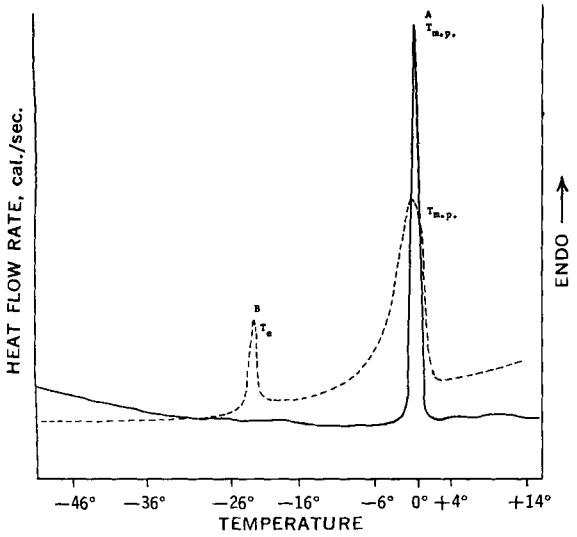

Figure 2—*Differential scanning calorimetry thermograms. Key: A, water; and B, 2.6% aqueous sodium chloride. Range = 1 and 8 mcal./sec. for A and B, respectively. Heating rate = 10°/min., and chart speed = 1 in./min.*

rate of 20°/min. The accepted value for the aqueous sodium chloride eutectic is −21.6° (1). Therefore, calibration of the temperature scale based on the endothermic transition of water resulted in a variation of 1.2° at the eutectic temperature of aqueous sodium chloride. The observed melting transitions ($T_{m.p.}$) of ice for 2.2 and 2.6% aqueous sodium chloride were −2.9 and +0.1°, respectively, with a deviation from calculated values of ±1.5°. Therefore, the observed variation in the differential thermal analysis transition temperature for water and aqueous sodium chloride was ±1.2–1.5°.

Sample Preparation for Differential Thermal Analysis—From 1 to 40 mg. of triturated samples was weighed into microsample tubes. Ten microliters of water was added to obtain samples containing 20–99% (w/w) water. The contents were carefully mixed with a needle, and then the tubes were plugged with a size 000 cork. After thorough equilibration, the samples were analyzed.

Calibration of Differential Scanning Calorimeter—The temperature scale was calibrated with water and evaluated with aqueous sodium chloride solution. The thermograms are shown in Fig. 2. Ten milliliters of test solution was added to the sample pan. The pan was capped, properly crimped, and transferred to the sample cell. A crimped empty reference pan was placed over the reference cell. The cell holder was covered with the flask (Dewar) and cooled to −85° by addition of liquid nitrogen. Samples were warmed under identical conditions at 10°/min.

The conventional procedure for determining melting points based on slope–baseline intercepts of a reference standard was not directly applicable. Variation in the inflection point and broad endothermic transition occurred in some cases. Therefore, the melting points were determined from the peak of each endotherm.

The endothermic maximum for the melting transition of ice corresponded to a dial reading of 107, and this value was assigned 0°.

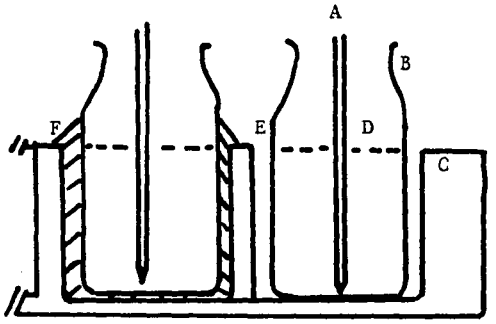

Figure 3—*Schematic diagram of samples in aluminum tray. Key: A, thermocouple; B, vial; C, aluminum tray; D, liquid level; E, air pocket; and F, vacuum grease.*

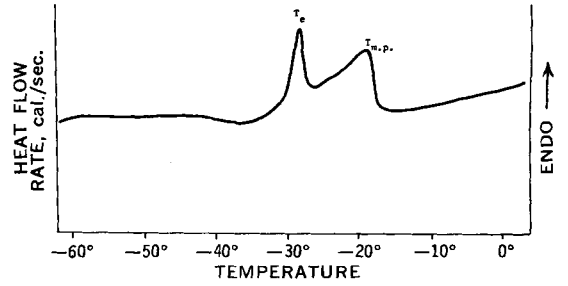

Figure 4—*Differential scanning calorimetry thermogram of System B with 9.8% sodium chloride and 56.71% (w/w) water. Range = 4 mcal./sec., heating rate = 10°/min., and chart speed = 1 in./min.*

A thermogram of dilute aqueous solution of sodium chloride (2.6%) showed a melting transition of ice ($T_{m.p.}$) at −1.5° and a sharp eutectic transition (T_e) at −22°. These values determined from the endothermic peaks were only 0.2–0.4° lower than calculated and literature values (1, 19). Therefore, calibration of the differential scanning calorimeter with water was considered adequate and resulted in small variations.

Thus, the differential scanning calorimetry procedure appeared comparable to the differential thermal analysis technique. As a result, both methods were used interchangeably throughout this study.

Sample Preparation for Differential Scanning Calorimetry—The sample pan and cap were tared on a microbalance. A triturated sample, 0.1–4 mg., and 1.0 μl. water from a microsyringe were added to the sample pan. The sample pan was then capped and crimped. Thus, samples containing 20–99% (w/w) water were prepared. Samples containing more than 80% (w/w) water were run immediately. However, those containing less than 80% water were left overnight to ensure equilibration of the contents. The equilibrated samples were checked for water loss and were found to retain weight within the error of the experiment. The samples were finally opened and inspected for homogeneous wetting.

Lyophilization Studies—An aluminum block, drilled to hold sample vials nearly equidistant, was used to assess heat conduction effects. Eight vials were placed directly into the block, while seven had the normal air space filled with high vacuum silicone grease[2]. This is shown diagrammatically in Fig. 3. The silicone was used in an attempt to improve heat transfer and thus reduce vial-to-vial variation. Two additional vials were placed directly onto the top shelf. In each vial, System A with 5.35% (w/w) added sodium chloride was dissolved in 7 ml. of water. Five thermocouples were placed in the silicone-packed vials and one thermocouple in an air-exposed vial.

The vials were equilibrated overnight to −50° and then brought to a higher temperature to obtain a desired percentage melt (*i.e.*, 0% melt at −38° or 14% melt at −25°). Four hours was allowed for final sample equilibration prior to the application of vacuum. During freeze drying, both the product temperature and shelf temperature were controlled to ±2° until sample thermocouple readings became irregular. The lyophilization was further continued at this lower shelf temperature for 24 hr. without attempting to control product temperature. Finally, the cycle was completed by heating to +50° at a rate of 5°/hr. The final products were compared for melt phenomena and overall physical appearance.

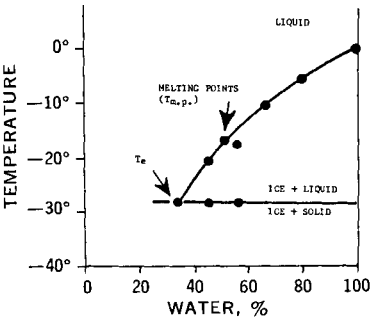

Figure 5—*Temperature–composition diagram of System B containing 9.8% sodium chloride.*

[2] Dow Corning.

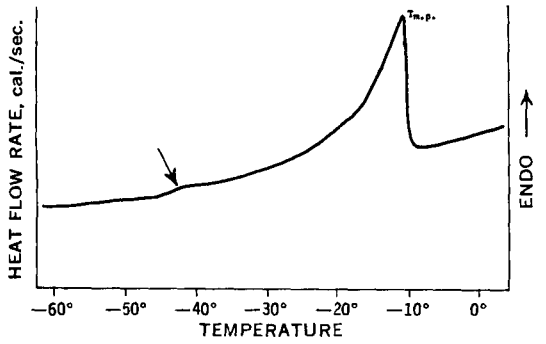

Figure 6—*Differential scanning calorimetry thermogram of System A with 5.3% sodium chloride and 66.66% (w/w) water. Heating rate = 10°/min., range = 4 mcal./sec., and chart speed = 1 in./min.*

RESULTS

Thermograms and Construction of Phase Diagrams—A differential scanning calorimetry thermogram for System B, containing 9.8% added sodium chloride and 56.71% (w/w) water is shown in Fig. 4. Both eutectic (T_e) and melting-point ($T_{m.p.}$) transitions were observed. The latter endotherm gradually disappeared as the water content in the sample reached that of the eutectic composition. Finally, only the eutectic transition was observed. A plot of transition temperature of ice against corresponding percent (w/w) water (Fig. 5) defines the melting-point curve and the eutectic temperature.

For several systems, A, B, C, and A with sodium chloride, the thermogram contained only one endotherm, the melting-point transition. A typical scan for System A with 5.35% added sodium chloride and 66.66% (w/w) water is shown in Fig. 6. It demonstrates a distinct endotherm due to the melting of ice ($T_{m.p.}$) and a subtle endothermic change at the inflection point (shown by the arrow). Similar endothermic transitions were also observed for this system containing varying amounts of water. However, with the reduction of water content, melting-point transitions shifted toward lower temperatures and endotherm intensity decreased. Finally, the endotherm disappeared for samples containing no freezable water. A plot of transition temperature of ice *versus* percent (w/w) water defines a melting-point curve, as shown in Fig. 7.

The eutectic and melting transitions were seen to coincide at the eutectic temperature for Systems B and C with salt (Figs. 5, 9, and 10). Therefore, the disappearance point of the endotherm approximates the eutectic temperature (T_e') for Systems A, B, C, and A with salt (Fig. 7). This approximation is affected by the amount of freezable water present (17) and the detectability of the endotherm associated with this water. Furthermore, these experimentally determined values appear valid since they were consistent with the results obtained under the controlled temperature lyophilization studies described later.

Percent Melt Calculation—The system described in Fig. 7 would have 100% melt above 0° but no melt below the apparent eutectic transition (T_e'). The percent melt at a given temperature would be calculated from $100X/X + Y$(12).

Melting-Point Curves for Various Systems—Data to describe melting-point curves were obtained under two experimental conditions. Each system, A, B, and C, was studied directly or with various amounts of sodium chloride. The melting-point curves generated from these various studies for Systems A, B, and C are shown in Figs. 8, 9, and 10, respectively.

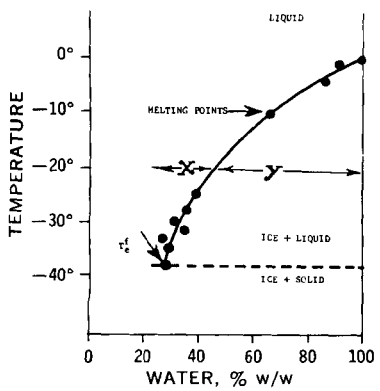

Figure 7—*Composition-temperature diagram for System A with 5.36% sodium chloride.*

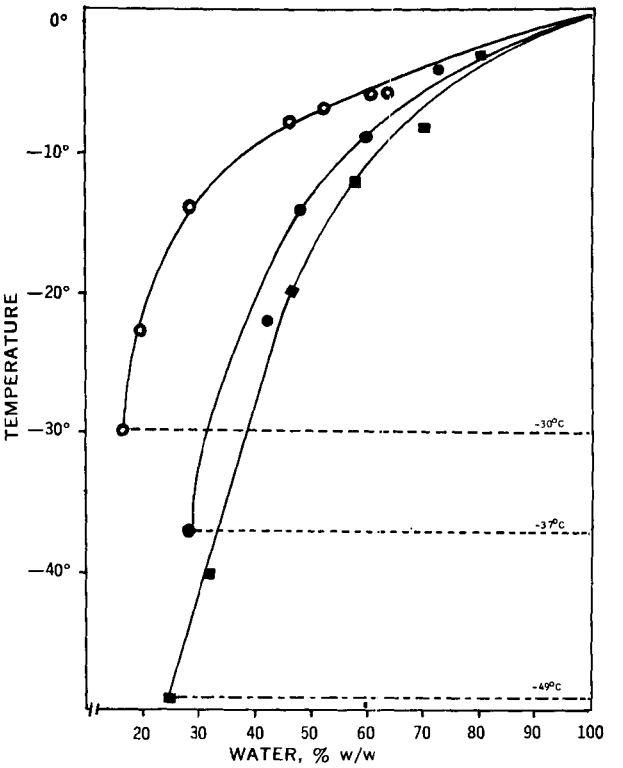

Figure 8—*Temperature-composition diagrams for System A with and without added sodium chloride. Key: ○, System A; ●, A with 9.6% sodium chloride; and ■, A with 13.8% sodium chloride.*

DISCUSSION

Formulation Variables—Lyophilization is a process used to overcome solution instabilities. Therefore, formulation components must be selected with reconstitution properties in mind. However, component selection and quantity also affect the acceptability of the physical properties of the dried mass as well as the time cycle required for lyophilization. The formulator must have reliable means to assess the effects of various additives. Components needed for bulking, buffering, or isotonicity also affect eutectic and thawing temperatures and, thus, optimum lyophilization conditions. As a result, several formulation combinations must be evaluated for a given drug.

Comparison of Systems without Salt—The melting-point curves obtained for Systems A, B, and C with no added sodium chloride appear in Figs. 8, 9, and 10, respectively. The apparent eutectic temperature for System A (T_e' −30°) was lower than that for System B (T_e' −22°). The addition of sodium acetate for buffering and the reduced lactose in System A were the probable causes. The eutectic temperature (T_e') of aqueous sodium acetate was found to be −28°. Sugars, on the other hand, showed widely varying results in reported studies (1, 4, 20). These observations on sugars range from glass formation with no discrete eutectic temperature (20) to little or no effect (1). In Systems A and B, there was no evidence of glass

Table I—Calculated Percent Melt Values for Systems A and C without Sodium Chloride

Temperature	Percent Melt	
	System A	System C
−20°	6.7	9.5
−22°	4.9	6.5
−25°	2.4	2.7
−29°	0.6	0.0(T_e')
−30°	0.0(T_e')	—

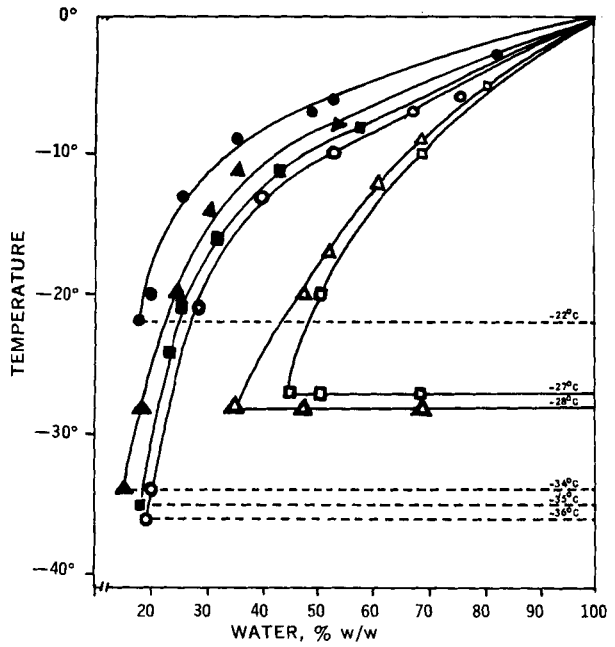

Figure 9—*Temperature–composition diagrams for System B with and without added sodium chloride. Key: ●, System B; ▲, B with 1.8% sodium chloride; ■, B with 3.5% sodium chloride; ○, B with 5.2% sodium chloride; △, B with 9.8% sodium chloride; and □, B with 15% sodium chloride.*

transition and the overall effect of lactose was to raise the eutectic temperature.

While the observed eutectic temperatures for Systems A and C were quite similar, significant overall differences were present in their melting point–composition diagrams shown in Figs. 8 and 10, respectively. The lower melting-point temperatures observed for System C probably result from the absence of lactose and the higher drug–buffer ratio in the composition. The extent of melt at temperatures near the eutectic point must be of concern since vial temperature within a freeze dryer may vary under production lyophilization conditions. Even though Systems A (−30°) and C (−29°) have very similar eutectic temperatures, they differ in the percent melt at selected temperatures. The comparison of percent melt values for these systems calculated from the melting-point curves (Fig. 8 and 10) are shown in Table I. The amount of melt at temperatures just above the eutectic temperature is greater for C than A. Thus, evaluation of proposed formulations should compare percent melt values at a given temperature as well as at the eutectic temperatures.

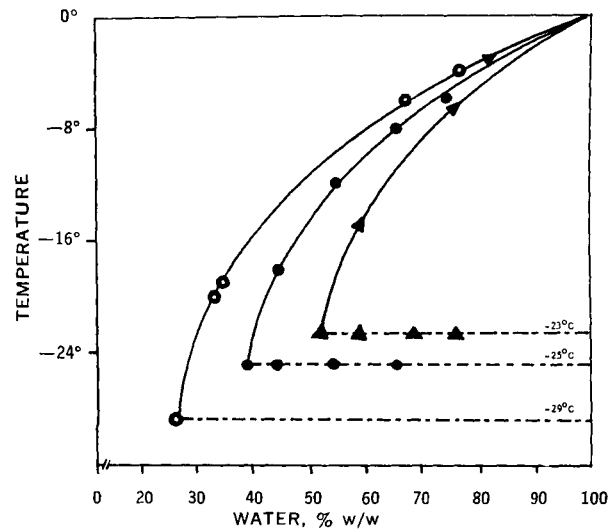

Figure 10—*Temperature–composition diagrams for System C with and without added sodium chloride. Key: ○, System C; ●, C with 7.7% sodium chloride; and ▲, C with 19.7% sodium chloride.*

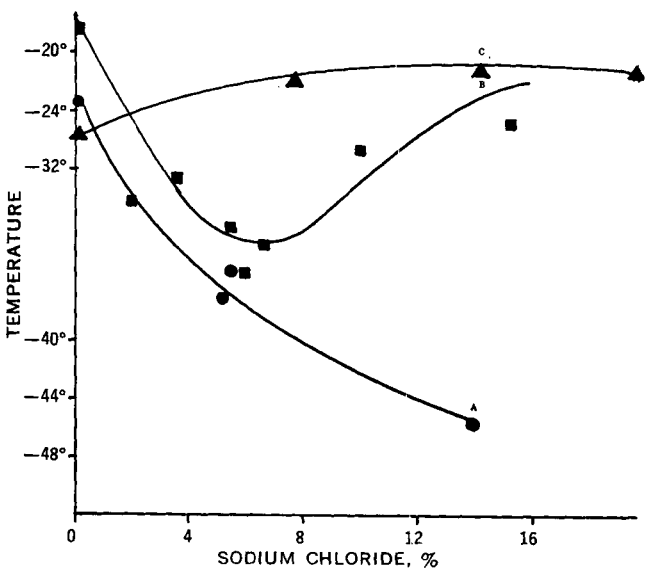

Figure 11—*Effect of added sodium chloride on melting points of various systems at 2.8% melt. Key: ●, System A; ■, System B; and ▲, System C.*

Comparison of Systems with Sodium Chloride—Sodium chloride was added to Systems A, B, and C to raise the eutectic temperature and improve the melt characteristics. The resultant apparent eutectic, true eutectic, and melting-point temperatures varied with the system and sodium chloride added. These observed effects on the melting-point curves are shown in Figs. 8–10.

For System A, increasing amounts of salt lowered both the apparent eutectic temperature (T_e') and the melting-point temperature along the curve (Fig. 8). Addition of up to 15% (w/w) sodium chloride in System A triturates resulted in the expected freezing-point depressions. However, no true eutectic transition was observed in any of these samples. Aqueous lactose solution with low levels of added sodium chloride did not show the eutectic transition of sodium chloride on differential thermal analysis thermograms. This apparent quenching effect was partly attributed to lactose in the composition. Masking of the sodium chloride eutectic transition by sodium acetate alone or in combination with lactose in System A could not be overcome. Therefore, no improvement in freezing-point characteristics nor any advantage for lyophilization resulted from sodium chloride addition to System A.

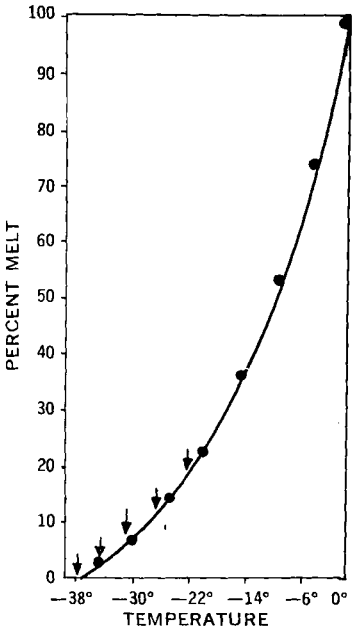

Figure 12—*Percent melt calculated from liquids for System A with 5.35% sodium chloride.*

The addition of 7.7% (w/w) sodium chloride in a triturate of System C resulted in the appearance of a true eutectic transition on the differential thermal analysis curve. The eutectic temperature was slightly higher than that for System C without sodium chloride. Also, added sodium chloride decreased the melting points and the percent melt at a given temperature. At −20°, the percent melt values for 0, 7.7, and 19.7% (w/w) added sodium chloride were 9.5, 5.0, and 4.2%, respectively. Thus, for System C the addition of sodium chloride may be expected to permit lyophilization at higher temperatures, resulting in a shorter cycle.

Small amounts of sodium chloride added to System B triturate produced behavior similar to that observed for System A. No true eutectic transition was observed. Both the apparent eutectic temperatures and melting points decreased, while percent melt at a given temperature increased. The apparent eutectic transitions (T_e') for 0, 1.8, 3.5, and 5.2% (w/w) added sodium chloride were −22, −34, −35, and −36°, respectively. However, addition of 9.8% (w/w) sodium chloride caused the appearance of true eutectic transitions similar to those observed for System C. These eutectic values were slightly lower than those for System B without salt (Fig. 9). The percent melt, which had initially increased, now decreased with added sodium chloride.

The data presented in Fig. 11 serve to summarize the various effects of added sodium chloride in these systems. The effects of sodium chloride on the melting points of Systems A, B, and C are shown at a fixed percent melt. Increased amounts of salt decreased the melting points for System A, increased those for System C, and initially decreased and then increased those for System B.

Formulation and Lyophilization Cycle Evaluation –The differential scanning calorimetry or differential thermal analysis melting-point diagrams supply the data necessary for selection of an optimum lyophilization formulation. A high eutectic temperature and low percent melt at temperatures near the eutectic are desirable characteristics. However, in some cases, a formulation possessing these optimum properties may not be suitable because of other requirements. These might involve solubility, volume of fill, desired final mass, isotonicity, buffering, or stabilization. A formulation not optimum on the basis of its melting-point properties could possibly be selected for some of these reasons. A formulation and its potential lyophilization cycle can be evaluated considering available eutectic temperature and percent melt data. Lyophilization trials can be designed to determine workable temperature ranges above the eutectic. The final evaluation of desirable cycle conditions would be based upon the physical appearance of the final dried product.

To test this procedure, a formulation of System A containing 5.35% sodium chloride was studied. This mixture did not exhibit optimal characteristics desirable for a lyophilization formulation. The mixture was a potential combination that might be needed to satisfy several diverse requirements. A melting-point diagram was generated (Fig. 7), and percent melt data were calculated from the melting-point curve. A plot of percent melt against temperature is shown in Fig. 12.

Separate lyophilization experiments were conducted at five different temperatures above the eutectic (−38 to −20°), representing varying amounts of melt (0–22.5%). The results indicated that:

1. To maintain temperature control within ±2°, it was necessary to immerse the sample vials in silicone. Vials placed directly into the holding block showed temperature variations up to 5–10° lower than desired during lyophilization, with the result that these vials exhibited much lower drying rates.

2. Acceptable cakes were obtained at −38 and −35°. However, at −35°, evidence of partial melt on the vial walls and some layering in the final cake were observed. These results supported the information derived from thermal analytical studies and the associated melting-point diagrams. The accuracy of the apparent eutectic temperature was substantiated in that no melt was observed at −38°.

3. As expected, with increasing melt, final cakes became less acceptable. Samples lyophilized at −30, −25, and −20°, representing 5.6, 14.1, and 22.5% melt, respectively, gave products with decreasing elegance.

4. It was concluded that this formulation could be lyophilized safely only up to 3° above its eutectic temperature. Any greater temperature difference would cause serious physical appearance deficiencies in the final cake.

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