

Lyophilization of Pharmaceuticals IV

Determination of Eutectic Temperatures of Inorganic Salts

By P. DELUCA and L. LACHMAN

The data illustrate that a relationship exists between the eutectic temperature and solubility of several inorganic salts. The greater the effect of temperature on solubility, the higher the eutectic point. The influence of the anion of various salts of a particular cation on the eutectic temperature is demonstrated. Through resistivity-temperature measurements, it is possible to determine the eutectic and freezing points on a single curve. The influence of an inorganic salt on the freezing and thawing characteristics of an antibiotic solution was demonstrated.

INORGANIC electrolytes are routinely used for the adjustment of tonicity in lyophilized injectable dosage forms. The effect that these inorganic salts can have on the freezing and drying characteristics of a medicinal agent should be considered in the design of freeze-drying cycles. This is especially true if the medicinal agent has a high eutectic temperature and the added salt possesses a significantly lower eutectic point. Such a combination would cause a change in the freezing and drying characteristics of the dosage form, generally resulting in a prolonged lyophilization cycle. Consequently, from the standpoint of elegance, time, and expense, it is essential to select the proper salt for adjusting the tonicity.

In an earlier investigation (1), preliminary data were reported illustrating that a relationship exists between the eutectic temperature and solubility of several inorganic salts. It was found that as the effect of temperature on solubility increased, the eutectic point temperature also increased. In this study, the effect of solubility and melting point on the eutectic temperatures of several inorganic salts was further investigated. In addition, the influence of an inorganic salt on the eutectic temperature of a medicinal agent was determined. The inorganic electrolytes selected for study were the halogen salts of sodium and potassium and the chlorides of lithium and cesium. The medicinal agent employed was an antibiotic salt commonly prepared as a lyophilized dosage form.

EXPERIMENTAL

Materials.—The inorganic electrolytes were of reagent grade material and were used as supplied from the vendors. All samples for testing were prepared with deionized water having a conductivity of 1×10^{-6} mhos/cm. or less at 25°.

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Resistivity Measurements.—Solutions containing 1.0 mole/L. of the electrolytes were employed for the resistivity measurements, except where the effect of concentration on the thawing pattern was studied. One milliliter of solution was transferred to the conductivity cell, and the thermistor probe and platinum electrodes were inserted just below the surface of the liquid. The cell was placed in a Plexiglas chamber, and the solution was frozen by allowing liquid carbon dioxide to flow through the cup holding the conductivity cell. By using a constricted inlet tube to the chamber, dry ice formed in the cup, thereby allowing a sample to be frozen to a temperature of -50° in less than 5 min. However, after discontinuing the flow of CO_2 , warming of the sample was gradual, requiring about 2 hr. to reach the freezing point of the solution.

A temperature-conductivity bridge was used to measure changes in resistance and temperature of the sample under investigation during freezing and thawing. These measurements were recorded on an X-Y plotter, using the 0–10⁸ ohm decade range on the conductivity bridge. This equipment was described in detail in an earlier paper (2).

RESULTS AND DISCUSSION

The temperature-resistivity curves are more easily interpreted for the inorganic electrolytes than for the organic compounds, since the phase changes which occur are more clearly defined. This sudden change of resistivity at the eutectic point results in an accurate measurement of the eutectic temperature. Although polarization appears to be evident with some of the inorganics, its effect is of little significance at the low temperatures employed.

The freezing and thawing curves for a 1.0 M solution of sodium chloride and the warming curve for pure water are illustrated in Fig. 1 by plotting the resistivity as a function of temperature. When the solution undergoes a phase change to the solid state, the resistance increases very sharply. The extent of supercooling is shown to be very significant for sodium chloride, with solidification occurring at about -30° . The true eutectic temperature, as seen from the warming curve in Fig. 1, occurs at -21.6° . These curves substantiate a previously reported observation (3) that the measurement of eutectic temperature during cooling would lead to an inaccurate determination. The eutectic temperature is obtained from the warming curve at the point where there is a sudden drop in

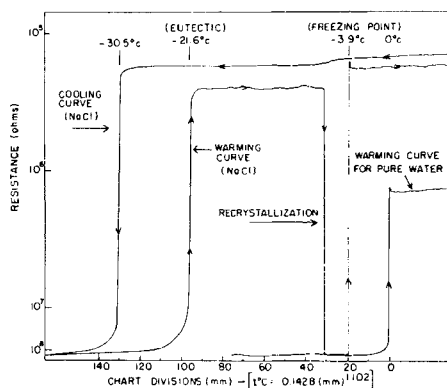


Fig. 1.—Resistance-temperature curves for the freezing and thawing of a 1.0 *M* sodium chloride solution.

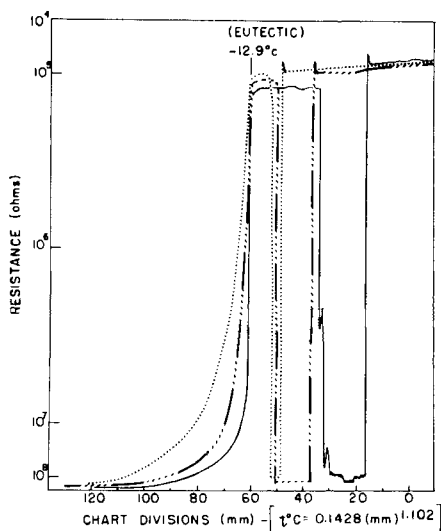


Fig. 2.—Influence of concentration on the resistance-temperature behavior of potassium bromide solutions. Key: —, 1.0 *M*; ·····, 2.0 *M*; - - - - - 3.0 *M*.

resistivity or, conversely, an increase in conductivity, due to the occurrence of liquid in the cell containing the frozen mass.

During the course of warming, a sharp increase in the resistivity has been observed above the eutectic point. The behavior is similar to what would be expected if the solution was to freeze suddenly. The resistivity of the mass remained very high until the freezing point of the solution was reached, at which temperature an abrupt break in the curve occurred. A review of the literature revealed that this phenomenon which occurs between the eutectic temperature and the freezing point is, in essence, a form of refreezing. Luyet and Rapatz (4), in their studies on the growth of ice and phase transitions in aqueous solution, used the term "recrystallization" to designate a crystallization that takes place in the course of warming frozen solutions. The recrystallization phenomenon will be discussed in a subsequent section of this report.

The effect of concentration on the conductivity behavior for several potassium bromide solutions is

illustrated in Fig. 2. The eutectic temperature was found to occur at -12.9° . The break in the curve at the eutectic point is sharpest for the 1.0 *M* concentration and most gradual for the highest concentration. The recrystallization phenomenon once again occurred at various locations between the eutectic temperature and freezing point of the solution. The break in the curve is suppressed toward the eutectic as the amount of electrolyte is increased. At each concentration, the mass appears to remain in this refrozen state until the freezing point of the solution is reached. The degree of supercooling for this salt was found to increase with increase in concentration. This effect is presently undergoing further investigation on several inorganic and organic salts.

The warming curves for 1.0 *M* solutions of sodium chloride, sodium bromide, and sodium iodide are illustrated in Fig. 3. The iodide salt exhibited the lowest eutectic temperature at -29.9° , followed by the bromide at -27.7° and the chloride at -21.6° .

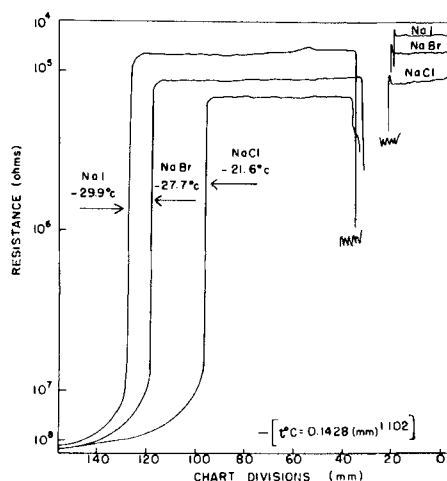


Fig. 3.—Resistance-temperature curves for the warming of frozen 1.0 *M* solutions of the halogen salts of sodium.

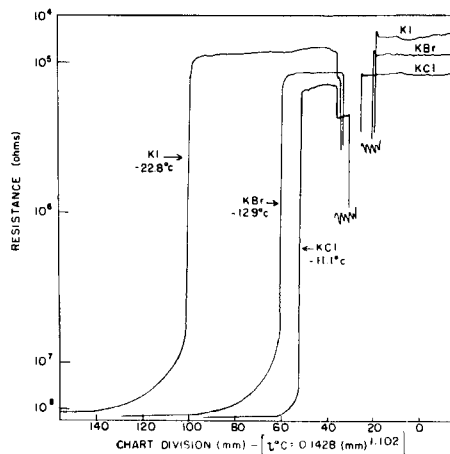


Fig. 4.—Resistance-temperature curves for the warming of frozen 1.0 *M* solutions of the halogen salts of potassium.

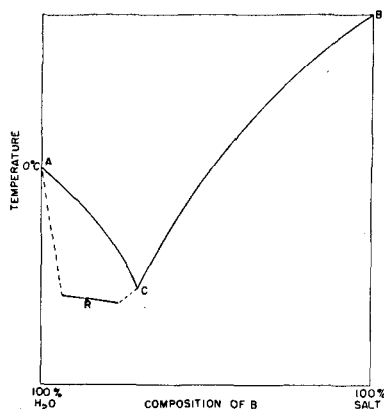


Fig. 5.—Phase diagram of a salt in water showing the area where induced recrystallization takes place.

The curves for 1.0 *M* solutions of potassium chloride, potassium bromide, and potassium iodide are illustrated in Fig. 4. As in the case of the sodium salts, the iodide salt of potassium exhibits the deepest eutectic point at -22.8° , followed by the bromide at -12.9° and the chloride at -11.1° . The change in eutectic temperature for the halogen salts is in accord with their positions in group VII of the periodic table. As illustrated in Figs. 3 and 4, at equimolar concentrations, the recrystallization phenomenon occurs at essentially the same temperature for the three salts of each cation.

In a thorough study on phase transitions at low temperatures, Luyet and co-workers (4-6) demonstrated the recrystallization process using photomicrographs of frozen electrolyte and glycerol solutions and, most recently, with motion pictures. The possibilities for the occurrence of recrystallization were discussed. One explanation offered for this occurrence is that when a solution is cooled rapidly so as to remain transparent, it suddenly becomes opaque when rewarmed rapidly. This change takes place within a relatively narrow range of temperature and has been termed "irruptive recrystallization" because crystalline particles become suddenly visible. Another explanation is that when a similar preparation is cooled slowly so as to contain crystals large enough to be seen under a microscope and then rewarmed slowly, the large crystals gradually grow larger, while the small ones disappear. This migrating growth of ice crystals caused by controlled or gradual warming has been referred to as "induced recrystallization." This

process has been demonstrated by Luyet to involve the complete loss of the original freezing pattern and the production of randomly distributed discrete ice crystals.

Figure 5 shows the phase diagram for a salt in water, illustrating that the area between line *R* and line *AC* represents the location where induced recrystallization takes place. From this phase diagram, it can be seen that for solutions prepared at concentrations to the right of the eutectic point, this phenomenon would not be evident. The warming curves for potassium bromide shown in Fig. 2 demonstrated that as the concentration of salt was increased, the recrystallization phenomenon occurred closer to the eutectic point. It has been observed that for solutions prepared at concentrations to the right of the eutectic point, the warming curves show a single break which corresponds to the eutectic temperature.

The eutectic temperatures and differential heats of solution, Lf' , for the inorganic electrolytes studied are listed in Table I. In addition, the literature and calculated eutectic values are listed for comparison.

The calculated values were determined using the expression previously developed for eutectic temperature.

$$\log \left(\frac{1}{T_e} - 0.0036616 \right) = - \frac{Lf'}{4.5758} \left(\frac{1}{T_e} - \frac{1}{T_0'} \right) - 2.8590$$

where

$$\begin{aligned} T_e &= \text{the eutectic temperature} \\ Lf' &= \text{differential heat of solution} \\ T_0' &= \text{melting point of the solid} \end{aligned}$$

The calculated eutectic temperatures, in most cases, show a close correlation with the experimentally determined values. This is a valid indication that the equation can be of considerable value in estimating eutectic temperature where sensitive conductivity equipment is not available for precise measurement. There is a general lowering of the eutectic temperature for the sodium and potassium salts corresponding to the lower melting points and/or Lf' values. In the case of the chloride and iodide salts of potassium, which melt at a similar temperature, the lower eutectic point for the iodide can be attributed to its solubility not being so greatly affected by changes in temperature. This is illustrated in Table I by the substantially lower differential heat of solution for the iodide salt.

The poor agreement between the calculated and experimental eutectic temperatures for lithium chloride can be partly attributed to the high solu-

TABLE I.—EUTECTIC TEMPERATURE AND SOLUBILITY DATA FOR SEVERAL INORGANIC ELECTROLYTES

Compd.	M. p., °C.	Lf' , ^a cal./mole	Eutectic Temp., °C.		
			Exptl.	Calcd.	Lit. Value
Sodium					
Chloride	801	880.9 ^b	-21.6	-24.01	-21.6
Bromide	755	716	-27.7	-29.76	-28.0
Iodide	651	650.9	-29.9	-33.09	-31.5
Potassium					
Chloride	776	1455	-11.1	-12.03	-10.7
Bromide	750	1431	-12.9	-12.72	-12.6
Iodide	773	802.2	-22.8	-26.67	-23.0
Lithium chloride	614	1036.1	Below -40	-21.57	
Cesium chloride	646	894.9	-24.1	-24.98	

^a Calculated from published solubility data (7). ^b Lf' value determined using published solubility data below 0.15°C .

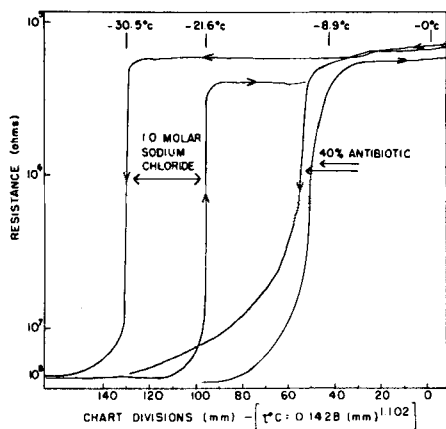


Fig. 6.—Resistance-temperature curves for the freezing and thawing of a 40% solution of an antibiotic and a 1.0 *M* solution of sodium chloride.

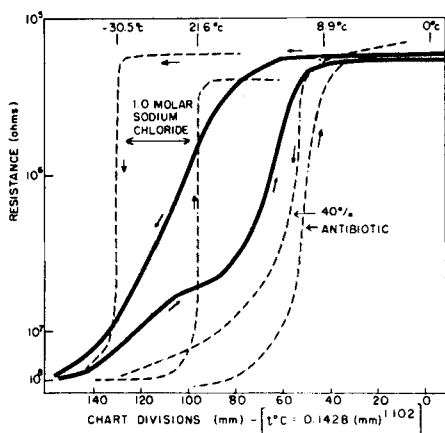


Fig. 7.—Resistance-temperature curves for the freezing and thawing of a solution containing 40% antibiotic and 1.0 mole of sodium chloride.

bility of this salt. The solubility varies from 20 molal at 25° to 16.3 molal at 0° which is equivalent to mole fraction values of 0.227 and 0.264, respectively. Since the development of the mathematical expression for eutectic values was based on mole fractions of 0.1 or less, the high mole fractions for lithium chloride would be expected to cause considerable error in the calculated eutectic temperature. In addition, over the temperature range used for the determination of the differential heat of solution, lithium chloride exists as a dihydrate. However, at -20° and lower, this salt has been reported to exist as a trihydrate and pentahydrate (8). This difference in hydration at the lower temperatures from the temperature range where the differential heat of solution was measured can contribute to the poor agreement between the calculated and experimental eutectic values for lithium chloride.

The cooling and warming curves for an antibiotic solution which is normally processed as a lyophilized product are shown in Fig. 6. The supercooling effect is very slight for a 40% concentration of this

compound with complete solidification occurring at about -10°. The eutectic temperature determined from the warming curve was found to be in the neighborhood of -8.9°. At this high concentration, there is no evidence of recrystallization occurring beyond the eutectic point. However, as observed in a previous study, this effect is not always evident for organic electrolytes because phase changes of these compounds are generally less pronounced than for inorganic electrolytes.

The effect of the addition of an inorganic electrolyte on the cooling and thawing curves for the antibiotic is demonstrated in Fig. 7. The degree of supercooling for the combination is less than that for a 1.0 *M* solution of sodium chloride, but greater than that for a 40% solution of the antibiotic. The warming curve for the mixture shows two breaks; one corresponds to the eutectic for sodium chloride, and the second occurs slightly below the eutectic for the drug. These breaks in the warming curves are not sharp, and the high concentration of drug appears to have significantly reduced the supercooling exhibited by the sodium chloride solution. The sharp break which normally occurs at the eutectic point of sodium chloride has been replaced by a plateau. It is evident from these results that serious consideration must be given to the influence an additive will have on the freezing and thawing properties of a compound intended for lyophilization. The curves illustrate that lyophilization of the pure antibiotic could be accomplished at a considerably higher temperature than would be possible if sodium chloride was added to the formulation.

On the other hand, if sodium chloride was considered as the medicinal agent intended for lyophilization, the addition of a substance which would reduce the degree of supercooling and alter the warming characteristics (Fig. 7) would result in considerable economy since the lyophilization could be carried out at a higher temperature and in a shorter span of time. As a result of the implications of these findings, a study was designed to evaluate the influence of additives as nucleating agents for purposes of diminishing the supercooling effect exhibited by many compounds.

SUMMARY

1. The freezing and thawing curves were presented for a series of inorganic electrolytes.
2. Eutectic temperatures were accurately determined from resistivity-temperature curves during the course of warming frozen salt solutions.
3. The influence of the anion species on the eutectic temperature of the cations sodium and potassium was evaluated. The gradual change in eutectic temperature for the halogen salts as the weight increases is in accord with their position in the periodic table.
4. The influence of melting point and differential heat of solution on the eutectic temperature was demonstrated.
5. The phenomenon of recrystallization which occurs between the eutectic and freezing points was discussed.
6. The effect of an inorganic salt on the freezing and thawing properties of an antibiotic was illustrated.

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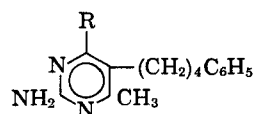
Analogues of Tetrahydrofolic Acid XXVII

Hydrophobic Bonding to Dihydrofolic Reductase by 2-Amino-4,6-disubstituted-5-alkylpyrimidines and 1-Alkyl-4,6-diamino-1,2-dihydro-*s*-triazines

By B. R. BAKER, BENG-THONG HO, and DANIEL V. SANTI

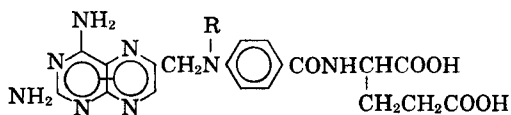
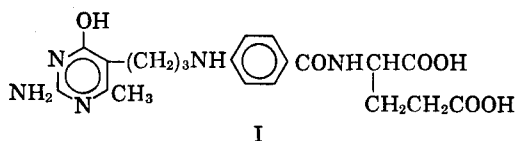
Strong hydrophobic bonding to dihydrofolic reductase has been observed by alkyl groups attached to the 5-position of pyrimidines or the 1-position of 1,2-dihydro-*s*-triazines. Studies were made with alkyl substituted 2,6-diamino-4-pyrimidinols (series A), 2-amino-6-methyl-4-pyrimidinols (series B), 2,4,6-triaminopyrimidines (series C), 2,4-diamino-6-methylpyrimidines (series D), and 4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazines (series E); 19 different alkyl, aryl, or aralkyl groups were investigated, but not in all series. Maximum hydrophobic bonding by an *n*-alkyl group was reached with *n*-butyl in all except series B; the B series required an *n*-amyl group. In all series, a further increment in binding over *n*-butyl was observed in the isoamyl group; the latter gave as good or better binding than the phenylpyrimidines or *s*-triazines of the pyrimethamine type. The butyl is probably complexed in a skew conformation. It is proposed that the 5-phenyl group of a pyrimidine or 1-phenyl group of 1,2-dihydro-*s*-triazine may also bind to dihydrofolic reductase by hydrophobic bonding. These observations on hydrophobic bonding can account for most of the discrepancies in increments of binding observed with the pyrimidines and dihydro-*s*-triazines in earlier papers. The possible uses of hydrophobic bonding to dihydrofolic reductase for species specificity are discussed.

AS A RESULT of a previous study (1), it was reported that 2-amino-6-methyl-5-(4-phenylbutyl)-4-pyrimidinol (VB) was a threefold better inhibitor of dihydrofolic reductase than the pyrimidyl analog (I) (2, 3) containing the intact *p*-aminobenzoyl-L-glutamate moiety. With the assumption that the phenyl group of VB is binding at the same locus on dihydrofolic reductase as the *p*-aminobenzoyl moiety of I—and there was no *a priori* reason why this assumption should not hold—it was predicted (4) that 2,4-diamino-6-phenethylpteridine (IV) should be as good an inhibitor of dihydrofolic reductase as the potent aminopterin (II) or amethopterin (III). Synthesis and evaluation of the phenethylpteridine (IV) (4) did not support this prediction. In fact, 2,4-diamino-6-methyl-5-(4-phenylbutyl)-pyrimidine (VD), which was about one-thirtieth



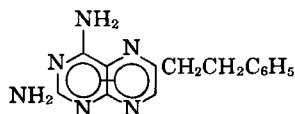
VB, R = OH

VD, R = NH₂



II, R = H

III, R = CH₃



IV

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Previous paper: Baker, B. R., and Jordaan, J. H., *J. Heterocyclic Chem.*, **2**, 162(1965).