

FREEZING POINTS OF ANTI-COAGULANT SALT SOLUTIONS

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(Accepted for publication, June 20, 1934)

In connection with the work of Ferguson (1) on the coagulation of blood, it was desirable to know the concentrations of solutions of the commonly used anti-coagulant salts, sodium citrate, oxalate, and fluoride, which possessed the same osmotic pressure as mammalian blood plasma or serum. Since freezing point data are given only for the fluoride in the International Critical Tables (2), we undertook to determine the freezing points of aqueous solutions of pure samples of these three salts in the range of physiological interest.

Materials

The sodium citrate was a high grade analyzed salt labelled $2 \text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 11 \text{H}_2\text{O}$, but was found to contain less than the theoretical amount of water. It was not possible to attain constant weight on drying either the crystals, or the residue obtained on evaporating a solution, at 150° or 180°C . Concentrations were finally determined by evaporating 10 ml. samples in porcelain crucibles at 110° and converting the citrate to sodium sulfate by the method used by Foote and Schairer (3) in the determination of sodium fluoride.¹ This is an indirect analysis for sodium. Our solutions of sodium citrate had a pH of about 9.0, a value at which the titration curve of Hastings and Van Slyke (4) indicates exact equivalence of sodium and citrate. Accordingly this salt was not further purified.

The sodium oxalate was an analyzed sample marked "Special, for standardizing according to Sørensen." It lost less than 0.1 per cent in weight on drying at 110°C . The purity of the dried samples was confirmed within 1 part in 1000 by titration with a potassium permanganate solution, 0.01442 M, which had been standardized against a high grade imported sample of oxalic acid. The concentrations of sodium oxalate in the freezing point experiments were determined by titrating 10 ml. samples with this potassium permanganate solution.

The sodium fluoride, according to the label, contained 0.5 per cent of free acid and 0.2 per cent of sodium fluosilicate. It was purified by neutralizing a saturated

¹The authors are grateful to Professor Harry W. Foote for his kindness in teaching them the technique of this useful analytical method.

solution to phenol red with carbonate-free sodium hydroxide, and filtering to remove fluosilicate. The fluoride was recrystallized by adding an equal volume of alcohol, heating almost to boiling, and cooling to about 2°C. The crystals were filtered off, air-dried, ground in a mortar, and dried at 110°C. The purified product was tested by conversion to sodium sulfate in platinum dishes according to Foote and Schairer (3). Four analyses agreed within 0.07 per cent, with an average deviation from the mean of 0.02 per cent. The average ratio of sulfate found to fluoride taken was 0.18 per cent lower than the theoretical. This result might indicate the presence of 0.18 per cent of an impurity volatile under the sulfuric acid treatment, or possibly as much as 0.33 per cent of sodium fluosilicate. The concentrations of the solutions in the freezing point experiments were determined by evaporating 20 ml. samples and drying to constant weight at 110°C.

Sodium chloride of a high grade analyzed brand was used in testing the freezing point technique. No analyses were made other than dry weight determinations, but the results of the freezing point measurements showed that the salt was sufficiently pure.

Method

The freezing points were determined by a method similar to that of Stadie and Sunderman (5), except that larger amounts of solution were used and the concentrations were determined by analysis or dry weight. Temperature was measured by a Heidenhain mercury thermometer with a fixed zero point and scale graduated in hundredths of a degree Centigrade. It could be read to the nearest thousandth with a magnifying glass. The freezing point vessel was a pint vacuum bottle without a case. It was kept in a well stirred, electrically controlled and refrigerated bath containing water and alcohol at $-0.50^{\circ}\text{C.} \pm 0.01^{\circ}$. The bottle was filled to about one-fourth of its capacity with crushed ice made from distilled water. This, together with about 50 ml. of water or solution, was sufficient to cover the bulb and 2 or 3 cm. of the stem of the thermometer. The zero point of the thermometer was repeatedly determined with ice and distilled water, and remained constant within 0.001°C. The mixture was gently stirred by lifting a glass ring stirrer a few times just before each reading was taken. Readings were made at 5 minute intervals, and the temperature was considered constant when three or four such readings agreed to 0.001°C. For the determinations with salt solutions, the water was siphoned off and replaced by about 50 ml. of solution. After thorough mixing of the ice and solution, the bottle was clamped in the bath and a dry pipette for taking the sample was inserted. Readings were taken as before until constancy of temperature was attained. A sample slightly greater than the amount to be measured was then removed and kept in a closed bottle. The mixture in the freezing point vessel was diluted by adding a little distilled water, and after thorough mixing readings were taken as before. This procedure was repeated to obtain freezing points of more dilute solutions. In some cases the order of the determinations was reversed by adding small amounts of a more

concentrated salt solution to a more dilute mixture. It was possible to check the zero point with the same ice which had been used for solutions, after washing it repeatedly in the bottle with cold distilled water. After the samples had reached 20°C., exactly 10 or 20 ml. were transferred by standardized pipettes to other vessels for the determination of the concentrations by the methods already described.

RESULTS

The results are presented in Fig. 1, in which the freezing point depressions, Δ , in Centigrade degrees, are plotted against the salt concentrations, C , in moles per liter of solution at 20°C. The points for each salt, in the range investigated, may be seen to fall close to a straight line. The lines were drawn on a large scale plot so as to make the positive and negative deviations nearly equal. The average deviations of the points from the lines are $\pm 0.002^\circ\text{C}$. The equations of these lines are as follows:

$$\text{Sodium citrate: } \Delta = 0.053 + 4.67 C.$$

$$\text{Sodium oxalate: } \Delta = 0.022 + 4.23 C.$$

$$\text{Sodium chloride: } \Delta = 0.004 + 3.415 C.$$

$$\text{Sodium fluoride: } \Delta = 0.002 + 3.39 C.$$

In the case of sodium citrate, the concentrations refer to moles of the tri-sodium salt, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, and not to the doubled mole sometimes used in formulas for the hydrated salt. The open circles on or near the citrate line represent solutions of sodium citrate in water at their natural pH value, about 9.0. The half-inked circles represent solutions of sodium citrate brought to pH 7.4 by the addition of very dilute hydrochloric acid. Within the limits of this method, there is no difference in freezing point due to the change in pH. The curve of Hastings and Van Slyke (4) indicates that citric acid is about 99.3 per cent neutralized at pH 7.4.

The solutions of the other salts were nearly neutral and had no appreciable buffer value. No attempt was made to adjust their pH values.

The half-inked circles near the sodium chloride line represent our determinations, and the line was drawn to represent these points. The fully inked circles represent determinations by Scatchard and Prentiss (6), who used a far more accurate method. We have recalculated their molal concentrations to moles per liter of solution at

20°. The fact that their points lie only 0.001 or 0.002°C. away from our line gives a measure of the reliability of our results.

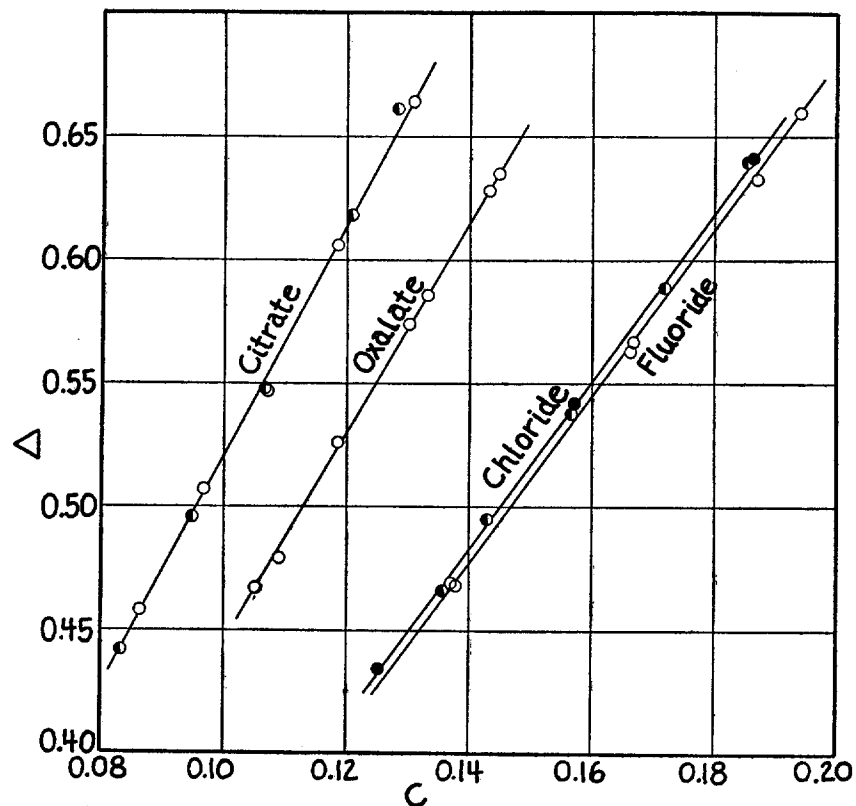


FIG. 1. Freezing point depressions of aqueous solutions of sodium salts plotted against concentration.

Δ = freezing point depression, °C.

C = concentration, moles salt per liter solution at 20°C.

○ = sodium citrate at pH 9.0, sodium oxalate, sodium fluoride.

◐ = sodium citrate at pH 7.4, sodium chloride.

● = sodium chloride, from Scatchard and Prentiss.

DISCUSSION

The data for sodium fluoride given in the International Critical Tables (2) are based on determinations by Peters (7). His points for

N/8 and N/6 sodium fluoride solutions fall about $0.025^{\circ}\text{C}.$ above our line. Since he used the Beckmann method, and gave no analysis of his salt, we believe our data to be more reliable.

Rous and Turner (8) reported that a 3.8 per cent solution of sodium citrate had the same freezing point as a 0.95 per cent sodium chloride solution. Leendertz and Gromelski (9) stated that a 1.55 per cent solution of sodium oxalate was isotonic with blood, as was a 3.55 per cent sodium citrate solution ($\Delta = 0.56^{\circ}\text{C}.$).

For purposes of comparison, the equations given above have been used to calculate the concentrations of the several salts which freeze at $-0.56^{\circ}\text{C}.$, according to our data. The concentrations are as follows:

- Sodium chloride, 0.1628 M, or 0.952 gm. per 100 ml.
- Sodium fluoride, 0.1646 M, or 0.691 gm. per 100 ml.
- Sodium oxalate, 0.1272 M, or 1.704 gm. per 100 ml.
- Sodium citrate, 0.1086 M, or 3.877 gm. of the higher hydrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5\frac{1}{2} \text{H}_2\text{O}$, per 100 ml.

It is to be emphasized that these concentrations are not given as those of solutions isotonic with average blood samples, but merely as those of solutions freezing at $-0.56^{\circ}\text{C}.$ Our equations make it possible to prepare solutions having a freezing point identical, within $0.002^{\circ}\text{C}.$, with that of any biological fluid, provided that the freezing point of the latter is accurately known and falls within the range studied. Freezing points of biological fluids may be determined by the method of Stadie and Sunderman (5).

SUMMARY

By a method involving equilibration of ice and solution, and analysis of the solution, freezing point depressions of solutions of sodium citrate, oxalate, and fluoride have been determined over the range $\Delta = 0.45$ to $0.65^{\circ}\text{C}.$ Determinations with sodium chloride solutions have confirmed the accuracy of the method. In each case the freezing point depression is given, within $0.002^{\circ}\text{C}.$, as a linear function of the concentration. By the use of these linear equations it is possible to prepare a solution of any of these four salts isotonic with a given biological fluid of known freezing point, provided the latter falls within the range studied.

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