

# RAPID CRYOSCOPIC TECHNIQUE FOR MEASURING OSMOTIC PROPERTIES OF DROP SIZE SAMPLES<sup>1, 2</sup>

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The measurement of osmotic pressure of aqueous solutions has been an indispensable tool for the physiologist. Such measurement has provided a great deal of information on the water relations of living tissues. Present methods, however, have the drawback of requiring relatively large samples and involving time consuming procedures.

The most common method of determining osmotic pressures is cryoscopy. The conventional cryoscopic technique, however, is rather slow, requires large samples, and the results require several corrections (3). In a variation of the conventional technique presented by Currier (4) sample size was reduced to about 0.7 cc and no corrections were necessary. This volume of sample is still rather large, however, and the measurements are rather slow. A commercially available thermistor (Aminco 5-2050) in place of the mercury thermometer permits a further decrease in sample size. This apparatus is costly, however, and the sample needed is still much larger than the drop size desirable for many studies. In 1913 Drucker and Schreiner (5) designed a cryoscopic technique using a microthermometer for drop-size samples, but the accuracy of their method is questionable.

The principal difficulty in measuring the freezing point of small samples is that of initiating freezing with a minimum of supercooling. With drop-size samples, supercooling becomes an insurmountable problem. Thus, measurement of the osmotic pressure of drop-size samples requires a method that does not involve supercooling. An accurate measurement of the vapor pressure of small samples, proposed by Baldes and Johnson (2) was modified by van Andel (1). The method involves measurement of the difference in temperature between a drop of sample and a drop of a known solution on separate loops of a thermocouple. A similar technique, based on the Peltier effect, was proposed by Spanner (7), and another by Richards and Ogata (6). All of these techniques are accurate but slow (about 30 min/measurement) and require rigorous temperature control ( $\pm 0.001$  C). Recently Weatherly (8) has proposed an empirical vapor pressure osmometer which does not require such stringent temperature control.

The problem of supercooling small samples can also be eliminated by freezing the solution and measuring the temperature during thawing. In the method given here a drop of solution is frozen on a thermistor and the temperature is recorded as the ice melts. The method uses simple equipment but allows rapid determinations of osmotic pressure or of molecular weight on drop-size samples.

## EXPERIMENTAL PROCEDURE

The experimental apparatus is presented schematically in figure 1. The sample chamber is at the tip of a glass tube with a ground-glass top. Orientation of the thermistor in the chamber is critical; the probe is placed in the same position inside the sample chamber by lining up the etched marks (indicated by arrows) on the probe and chamber. The thermistor is one of four legs of the Wheatstone bridge. A

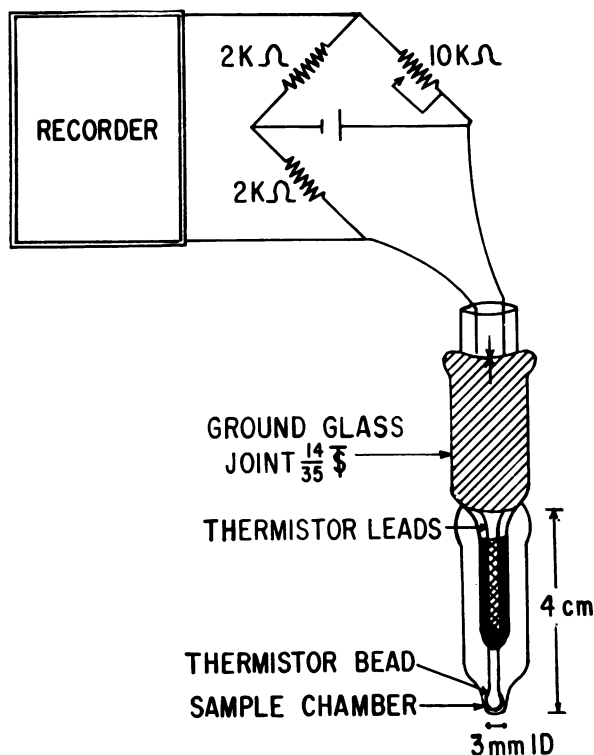


FIG. 1. Schematic diagram of the experimental apparatus. The thermistor used is  $2k\Omega$  at 20 C (Veco 32A1).

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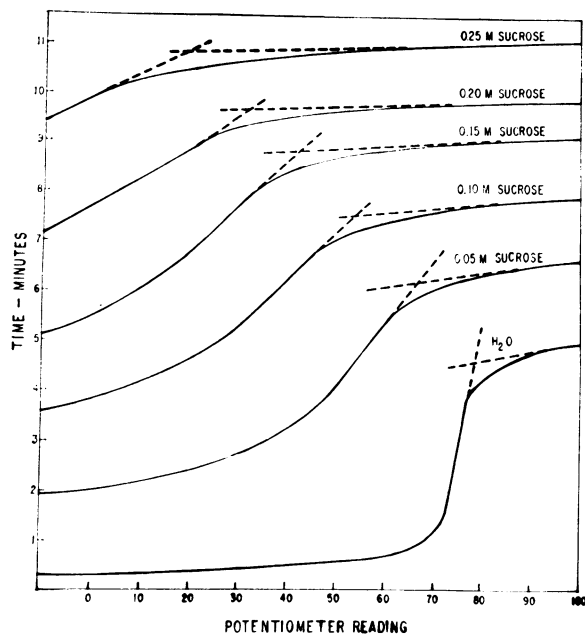


FIG. 2. Tracing of the potentiometer chart illustrating the temperature-time functions for various sucrose concentrations, and the manner of drawing tangents.

potentiometer records imbalances in the bridge that result from changes in resistance caused by changes in temperature. Mercury cells of 1.5 v are a source of constant emf.

The analytical procedure is as follows: A drop of the sample is placed in the chamber, the probe is aligned and inserted into the chamber, and the tip of the tube is immersed in a mixture of alcohol and dry ice, freezing the sample instantly. The tube is removed from the freezing mixture and wiped dry, and the sample is allowed to warm slowly in air above a water-ice mixture in a narrow-mouth Dewar flask. The temperature of the sample during thawing is recorded as a function of time on the potentiometer chart.

Results obtained for a series of sucrose solutions are presented in figure 2. The change of slope of the curves from the heat of melting is quite evident. First, the slope is rather flat, indicating a rapid temperature increase. The temperature rise slows as the melting point is approached, and becomes rapid again when all ice in contact with the thermistor bead has melted. The slow change of temperature of the ice during melting is due to the latent heat of melting. The slope of the curve at this region is quite steep for pure water, becoming less so with increasing solute concentrations. At higher solute concentrations the temperature of ice during melting changes much more slowly than the temperature of water or dilute solutions. The difference corresponds to a wide variation in the concentration of the solute in the liquid phase as melting proceeds. The success of the method depends on direct measurement of the ice tem-

perature, which requires that the ice be in direct physical contact with the thermistor bead. If the ice separates from the thermistor prematurely, the concentration of solute will be seriously overestimated. For this reason (fig 2) the method is limited in its range to solutions no more than 0.25 osmolal.

In computing the osmotic pressure, lines are drawn tangent to the slope during melting and the slope after melting (fig 2). The point of intersection of the tangents for different sucrose concentrations is subtracted from the point of intersection for pure water and the difference is plotted in a calibration curve. In this manner the intercept of the calibration curve is zero.

Figure 3 is a calibration curve plotted from the data in table I. From such a calibration the osmotic pressure of unknown aqueous solutions can be determined by measuring the temperature during thawing. After the point of intersection of tangents is established, the osmolality of an unknown can be read directly from the calibration curve.

The method can be used quite effectively in determining the atom percentage deuterium in water. This method utilizes the fact that the freezing point of pure  $D_2O$  is 3.82 C and decreases linearly to the freezing point of water with decreasing atom percent deuterium. Figure 4 illustrates a calibration curve for the determination of atom percentage deuterium. In this case, of course, the measurement can be much more accurate than in the case of solutes because the differences in physical properties of the solute (DHO) and the solvent are relatively minor.

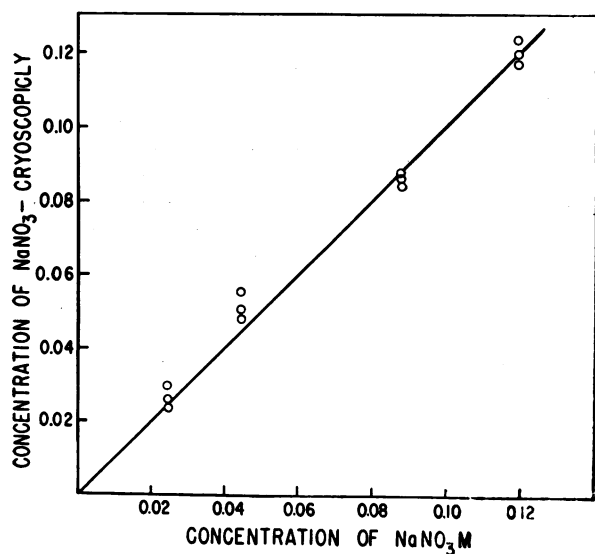
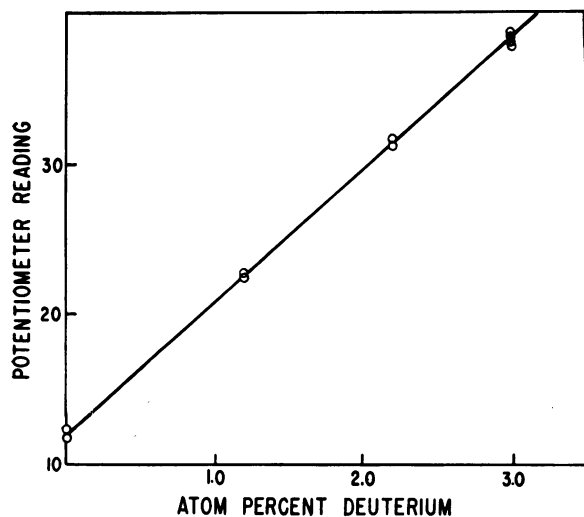
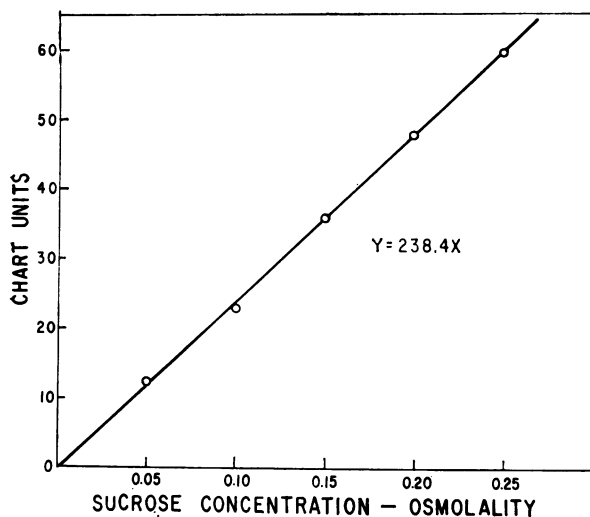
The method is not confined to water as a solvent. We have used it successfully in determining the molecular weights of organic compounds dissolved in benzene. This is done by determining a calibration curve for a benzene solution comparable to that for aqueous solutions.

Several precautions must be taken to ensure optimal performance of the method:

TABLE I  
EXPERIMENTAL RESULTS FOR SUCROSE CALIBRATION  
CURVE AS OBTAINED BY DIFFERENT OPERATORS

SUCROSE CONC OSMOLAL	OPERATOR				MEAN
	1	2	3	4	
0.05	12.3*	12.1	12.6	12.8	12.4 ± 0.27
0.10	23.0	22.0	23.6	23.2	22.9 ± 0.66
0.15	36.7	35.3	35.9	35.5	35.9 ± 0.54
0.20	47.7	48.4	47.9	46.9	47.7 ± 0.54
0.25	58.6	61.5	60.4	58.8	59.8 ± 0.91
Slope	237.1	241.0	240.4	235.4	238.4
99 % confidence limits of slope 238.4 ± 5.34					
95 % confidence limits of slope 238.4 ± 3.19					
90 % confidence limits of slope 238.4 ± 2.70					

\* Displacement of tangents' intersection from that of pure water in chart units



I. The thermistor *must* be placed centrally in the same chamber, and the gap between the bead and the chamber wall should be as small as possible.

II. The volume of the sample is critical. During thawing the ice must separate from the thermistor uniformly. Excess volume of sample above the bead will interfere with the measurement.

III. A constant voltage supply eliminates any need for frequent calibration.

IV. For best results, rate of thawing should be slowed by keeping the sample over an ice bath.

V. The external surface of the sample chamber should be wiped dry before thawing.

#### EVALUATION OF METHOD

The chief difficulty expected was that of drawing tangents. Three workers were therefore requested to determine a sucrose calibration curve to test the magnitude of error (table I). There is no significant difference between operators. The standard error for a single determination is  $\pm 0.003$  (0.067 atm) and  $\pm 0.007$  ( $\pm 0.157$  atm) for 0.05 and 0.25 osmolal respectively. For triplicate determinations the standard errors are  $\pm 0.001$  (0.022 atm) and 0.004 (0.090 atm) for the same concentrations.

Another test of the accuracy of the method is presented in figure 5. The concentrations of sodium nitrate solutions were determined cryoscopically. Figure 5 compares the measured and the known values.

Although benzene has a much lower latent heat of melting than water, it can be used as a solvent in this method. A calibration was established for solutions of benzoquinone in benzene. The molecular weight was determined for a synthetic organic product, presumably the trimer of  $\alpha$ -picolene. To verify whether the desired product was obtained, the molecular weight of a sample of the known dimer of  $\alpha$ -picolene (MW 184) was 183. The molecular weight of the presumed trimer was 373, indicating that the product was probably the tetramer (MW 368) rather than the trimer (MW 276).

From the evidence presented, it is concluded that the proposed method is reliable, rapid, and suitable for measuring the osmotic pressures of dilute drop-size samples.



FIG. 3 (*top*). Sucrose calibration curve—temperature (in arbitrary chart units) as a function of sucrose concentration or equivalent osmotic pressures (conversion of concentration to osmotic pressure taken from the critical tables).

FIG. 4 (*center*). DHO calibration curve—temperature in arbitrary units as a function of DHO concentration.

FIG. 5 (*bottom*). Comparison between known and measured concentrations of NaNO<sub>3</sub>.

## SUMMARY

A rapid cryoscopic method proposed for drop-size samples consists of freezing a drop of solution and following its temperature during thawing with a thermistor and a potentiometer chart. The method can be used for determining osmotic pressure, the molecular weight of solutes, and the concentration of deuterium in heavy water.

The method is limited to dilute solutions (up to 6 atm osmotic pressure) and is sensitive to better than 0.1 atm.

## LITERATURE CITED

1. ANDEL, O. M. VAN. 1952. Determination of the osmotic value of exudation sap by means of the thermoelectric method of Baldes & Johnson. Proc. Kon. Ned. Akad. Wetensch. C. 55: 40.
2. BALDES, E. J. & A. F. JOHNSON. 1939. The thermoelectric, its construction & use. *Biodynamica* 2(47): 1-11.
3. CRAFTS, A. S., H. B. CURRIER, & C. R. STOCKING. 1949. Water in the Physiology of Plants. *Chronica Botanica*, Boston, Mass.
4. CURRIER, H. B. 1944. Cryoscopy of small amounts of sap tissue. *Plant Physiol.* 19: 544-550.
5. DRUCKER, C. & E. SCHREINER. 1913. Mikrokryoskopische Versuche. *Biol. Zentral.* 33: 99-103.
6. RICHARDS, L. A. & G. OGATA. 1958. Thermocouple for vapor pressure measurement in biological & soil systems at high humidity. *Science* 128: 1089-1090.
7. SPANNER, D. C. 1951. The Peltier effect & its use in the measurement of suction pressure. *J. Exp. Botan.* 11: 145-168.
8. WEATHERLY, P. E. 1960. A new micro-osmometer. *J. Exp. Botan.* 11: 258-268.

EFFECTS OF THERMOPERIODISM ON TUBER FORMATION IN  
IPOMOEA BATATAS UNDER CONTROLLED CONDITIONS<sup>1</sup>YONG CHOLL KIM<sup>2</sup>

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Went (9) and Viglierchio and Went (8) found that the night temperature is the most critical factor governing the developmental process in tomato, chili pepper, tobacco, and beans. The importance of night temperature in general to plants was also suggested.

Previous work on *Ipomoea batatas* indicated that tuber formation is a process depending more on the condition of the growing point than on carbohydrate production in the leaf (6). Later a sensitivity to photoperiodism was found (7).

With respect to temperature, only some general effects on tuber formation have been reported.

Thus, Isiguro (5) found that with an average temperature less than 20 C, there was a decrease in yield, and Ido (4), that high temperature favors tuber production. Dogari (2) indicated that tuber production might be adapted in low soil temperature of 19 to 21 C. However, to date, there is no information on the effect of thermoperiodism on tuber formation. The following experiments, therefore,

were undertaken under controlled conditions to study the effects of low night temperature, etc., on tuber formation as well as growth.

## MATERIALS &amp; METHODS

The experiments were conducted in thermoperiodically-controlled rooms, with a photoperiod of 16 hours and 8 hours of darkness in all cases. Incandescent lamps, providing a light intensity of approximately 100 to 150 ft-c were used.

The environmental factors may be divided into two general groups, nutritional and others. Since growth depends on a number of factors, it was possible to obtain undersized plants by restricting several, such as quantity of nutrition and light. This was advantageous for these experiments both from a standpoint of using a large quantity under uniform conditions, as well as easy handling for harvesting, with laboratory scales. Satisfactory tuber formation was obtained under the restricted conditions.

The seed tubers of *Ipomoea batatas* var. Okinawa 100, were sprouted in sand in wooden boxes and grown at the given temperature of each experiment. When the young plants were about 20 cm high, uniform cuttings of 8 to 10 cm, containing four nodes

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