# Microcryoscopy of Water Employing Silver Iodide to Break Supercooling

Tetsuya Ogura,* Maria Lorenza Salinas,†† Cynthia Genoveva Quiroz, Hiroyuki Miyake,† and Yoshitane Kojima†
*Departamento de Quimica, Universidad Autonoma de Guadalajara, AP 1-440, Av. Patria No. 1201,*
*Lomas del Valle, Guadalajara, Jalisco, Mexico CP 44100*
†*Department of Chemistry, Graduate School of Science, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558-8585*

(Received December 25, 1998; CL-980956)

A microcryoscope for water has been successfully developed. A series of experiments using 25 μL samples were presented here. It was shown that microcryoscopes can be small sized employing a smaller thermistor and a folded Al-foil as container. AgI was employed to promote ice nucleation.

Figure 1 shows a typical cooling image in a cryoscopic measurement of water. The ordinate indicates time course while the abscissa shows cryoscope temperature measured by use of a thermistor. The freezing point is determined experimentally on the basis of the plateau *D*.[1] The freezing point decreases proportionally to the numbers of solute particles such as molecules and ions per unit volume, while the proportionality constant can be evaluated on the basis of the latent heat of freezing. Therefore, the total number of solute particles in a solution can be counted by simply determining the difference in freezing points between the pure solvent and the solution. This constitutes a specialized tool for measuring the number of solute particles.

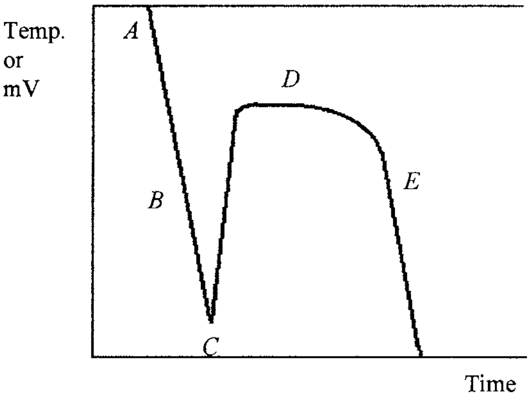

**Figure 1.** The temperature behavior in cryoscopy. *A*: initiation of cooling. *B*: supercooling. *C*: breaking point of supercooling. *D*: leveling-off plateau. *E*: cooling of ice.

The liquid temperature decreases from the moment the sample is placed in a cold bath (indicated in Figure 1 as *A*), and continues to decrease to a point far below 0 °C (indicated as *B*, supercooling). The supercooling halts abruptly at point *C*, from which the curve reflects increasing temperature until a levelling off point is reached (*D*), which is followed by a period of rapid cooling (*E*).

*A* and *B* are cooling curves of liquid water. Ice nucleation takes place at *C*, releasing the latent heat of freezing which pushes up the temperature in accord with ice formation until the ice-water equilibrium temperature (0 °C) is reached. The slight temperatue drop at *D* might be due to increasing concentrations of impurities or solutes over time resulting from consumption of pure water by ice formation. *E* indicates a cooling curve of ice after all water has been frozen.

Water resists freezing even at a temperature far below the freezing point. Jeffery and Austin stated that a series of laboratory and aircraft measurements indicated that supercooled liquid water exists at temperatures as low as -70 °C.[2]

Plants can survive temperatures as low as -10 °C. On the other hand, a colonization of a bacteria, *Pseudomonas syringae*, in plant leaves provokes freezing at a much higher temperature, -3 °C, causing frost damage since ice destroys the cell wall.[3] It is reported that water in a 100 mL beaker freezes in a range of -5 to -20 °C,[4] which corresponds to the supercooling that takes place in cryoscopy employing a glass vessel.

We have aimed at developing a microcryoscope suitable for biological specimens that are only available in volume of 10-50 μL. Let us consider a cryoscopic experiment in which the following conditions apply: the cryoscope consists of a 33 μL cylindrical glass vessel (3 mm i.d. x 4.7 mm depth, flat bottom) with 1 mm of wall thickness filled with 25 μL of water and 8 μL of the glass portion of a thermistor, the composite is cooled in a cold bath, the supercooling is broken at -10 °C, and the leveling-off plateau is reached at a composite temperature of 0 °C.

Assuming that the composite is cooled homogeneously and that the heat capacity and density of the glass are the same as those of quartz, i.e., 0.18 cal/(g·deg) and 2.2 g/mL,[5] respectively, the heat capacity of the composite is estimated as (78.6 + 8) μL x 0.18 cal/(g·deg) x 2.2 g/mL, or 0.034 cal/deg. Thus, approximately 0.34 cal is consumed to raise the composite temperature 10 °C. The water also consumes 0.25 cal. Accordingly, 0.59 cal is required in total. On the other hand, complete freezing of 25 μL water releases approximately 2.0 cal. Hence, roughly 30% of the water must be converted to ice in the process to reach 0 °C.

The supercooling breaking point is important since it informs us of the moment that ice formation begins. However, if the breaking points differ by 1 °C in two cryoscopic runs employing the same solute concentration, the concentrations of a solute at the plateau might differ by 3%. Consequently, it is indispensable to control the break point with precision considerably greater than 1 °C. We found silver iodide employed for seeding of rainfall[6-10] is quite effective in breaking supercooling and that the break point falls into a range that does not vary by more than 0.2 °C if the cryoscope is placed in a cold bath kept at -5 °C.

The cryoscopic cooling is a dynamic process in which heat is removed mainly from the surface of the vessel, while heat is added to the system by the thermistor current. It is important that the cold bath temperature be kept constant. An addition of 20% aqueous ethanol solution to crushed ice in thermos results in a bath temperature of approximately -5 °C, and the temperature is kept constant for a suitably long period

We faced a strange problem: The temperature plateau was highest in the first measurement and decreased with each subsequent run. Vessels of different materials and sizes were substituted with no change in results. Improvements were obtained by altering thermistor, especially changing the type. However, the tendency toward lower temperatures could not be eliminated.

Thermistor current was passed just before introduction of the cryoscope to the cold bath. When the plateau was reached, the thermistor current was cut. Then, the cryoscope was taken out of the bath and the outer tube was removed. The composite was warmed by a mild flow of air at room temperature for 10 min or more before being employed for the next measurement. Acceptable reproducibility was achieved. The hysterisis would surely be lessened using thinner walled thermistors.

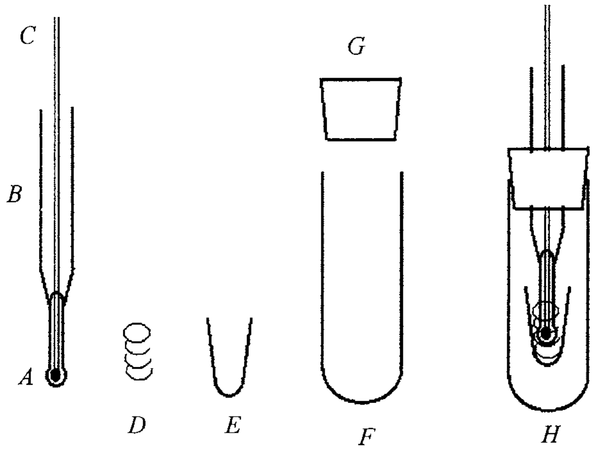

**Figure 2.** Construction of the microcryoscope. *A*: sensor. *B*: thermistor holder. *C*: cables. *D*: Ag wire. *E*: container. *F*: outer tube. *G*: rubber stopper *H*: entire cryoscope.

Figure 2 shows the construction of the cryoscope. A glass sealed thermistor (Fenwal Electronics, cat. no. 120-103FAJ-001), *A*, was glued with epoxy resin to a glass supporting tube, *B*. A thin silver wire (0.1 mmϕ), *D*, was spiraled around the temperature-sensing portion and continued 1.5 mm beyond the thermistor tip in order to attain a homogeneity of solution temperature. The Ag wire was reacted with $I_2$ in benzene to form AgI.

A commercially available Eppendorf centrifuging tube (500 μL, polypropylene) was cut off approximately to 5 mm height and the bottom part, *E*, was employed as the container.[11] Around 1 $mm^3$ of ground AgI (Wako Pure Chemicals) was daubed on the inner wall of *E* and excess AgI was shaken out. Then, a 25 μL aqueous solution was pipetted into *E* and the container was affixed to the thermistor using adhesive tape. The composite was placed into the outer tube *F* with the aid of a rubber stopper *G* to form the entire cryoscope *H*.

Cables *C* derived from the thermistor were connected to a Wheatstone bridge and the off-balanced bridge potential was registered by a recorder in order to measure the temperature change.

Sucrose solutions ranging to 0-0.1 M were measured by the microcryoscope as shown in the graphic abstract.[12] These data are expressed by a linear relation using least square method as follows:

$$\text{mV reading} = 36.81 \times \text{conc. (M)} + 0.12 \pm 0.11$$

where the error term is standard deviation and the intercept comes from an artificially-adjusted bridge balance. The statistic linear correlation factor is evaluated to be 0.997.

As seen in the graphic abstract, the measured values were accompanied by a certain degree of error which must be lessen by further studies. It is recommendable to average duplicated or triplicated measurements.

The reduction of heat capacity of the sensor and container must permit to reduce sample size without affecting the shape of Figure 1. Cryoscopes have been constructed to operate with microliter-order sample employing a smaller thermistor and a folded Al foil as container.

This research was supported by the Consejo Nacional de Ciencia y Tecnologia (0512-9108) and Tequila Cuervo, SA de CV, Mexico. We are grateful to Osaka City University Fellowship for Foreign Researchers (1998) awarded to TO.

## References and Notes

[††] This study was done to fulfill a part of the requirement to acquire the master's degree from the Universidad de Guadalajara.

1 Most of textbooks recommend to extrapolate the plateau *D* and use the cross-point to the cooling curve A-B as the freezing point (eg. D. P. Shoemaker, C. W. Garland, and J. W. Nibler, "Experiments in physical chemistry." 6th ed., McGraw-Hill, New York (1996). We plotted the highest temperature or the largest mV value at *D* as the freezing point in Figure 2.

2 C. A. Jeffery and P. H. Austin, *J. Geophys. Res. Atmos.*, **102**. 25269 (1997).

3 M. R. Warmund and J. T. English, *J. Am. Soc. Hortic. Sci.*, **123**, 234 (1998).

4 D. Auerbach, *Am. J. Phys.* **63**, 882 (1995).

5 "CRC Handbook. Chem.," ed by D. R. Lide, CRC Press Inc., Boca Raton, Fl (1993), pp 12-165.

6 B. Vonnegut, *J. Appl. Phys.*, **18**, 593 (1947).

7 I. Langmuir, " General Electric Res. Lab. Publ. No. RL 361," Schenectady, N. Y. (1950), p. 20.

8 V. J. Schaefer, *Bull. Am. Meteorol. Soc.*, **19**, 337 (1963).

9 D. C. Blanchard, *Bull. Am. Meteorol. Soc.*, **27**, 1279 (1996).

10 W. G. Finnegan, *J. Colloid Interface Sci.*, **202**, 518 (1998).

11 The milivoltage readings at different concentrations were as follows: 0 M ; 0.01 mV. 0; 0.096. 0.02; 1.124. 0.02; 0.974. 0.04; 1.66. 0.04; 1.65. 0; 0.006. 0; 0.008. 0.08; 3.01. 0.08; 3.035. 0.06; 2.22. 0.06; 2.365. 0.1; 3.80. 0.1; 3.77.

12 The polypropylene container weighed approximately 0.03 g. Since the heat capacity is 0.44 cal/g (J. Brandrup and E. M. Immergut, "Polym. Handbook," 3rd ed, John Willy & Sons, Inc. (1989), p V29.), the container consumes 0.13 cal to be warmed up 10 °C, on the other hand, the glass vessel was estimated to be 0.31 cal.