Freezing Point of Milk: A Natural Way To Understand Colligative Properties

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Laboratory exercises using natural systems catch students' interest and can integrate important physicochemical concepts. For example, fluid compartments in humans and most animals are iso-osmotic. The freezing point of blood serum is very nearly the same as that of the cerebro–spinal fluid despite their very different compositions. Therefore, these natural fluids are good examples of colligative properties.

We describe a laboratory exercise dealing with freezing point depression of milk that illustrates application of this colligative property from the analytical and the physicochemical points of view. Since milk is a mixture of solutes in aqueous solution, this experiment helps the students to understand that the contribution of each solute depends only on its concentration and not on its size or mass. Moreover, milk contains suspended fat particles and colloidal proteins that do not contribute to freezing point depression *(1).* By comparing whole milk and skim milk the students can better understand the differences between dissolved and suspended particles.

The first part of the exercise illustrates a quality test of milk based on freezing point measurements, which is an approved, worldwide method to test for adulteration by water addition. The second part of the experiment determines the effective molar mass of milk, a typical chemical application of the freezing point depression technique.

Measurement of Freezing Point Depression

The conventional method for measuring freezing points is tedious *(2, 3)*. We use instead a Fiske osmometer (Fiske Associates), a widespread, reasonably priced instrument that allows an easy and fast measurement of freezing point depression of aqueous solutions. A typical measurement takes about 90 seconds, and usually small quantities of sample are needed. Fiske osmometers give values of osmolality (Osm), a measure of the total concentration of osmotically-active particles in a solution equal to the sum of the molalities of all dissolved particles. In an aqueous solution 1 Osm causes a freezing point depression of 1.86 °C, so that osmolality values can be directly converted into freezing point values using

$$T_f = T_f^* - \left(1.86\,\frac{°\mathrm{C}}{\mathrm{mol\ kg^{-1}}}\right)\left(x\ \mathrm{mol\ kg^{-1}}\right) \qquad (1)$$

where T_f is the freezing point of the solution, T_f^* is the freezing point of the pure solvent (in our case water, so $T_f^* = 0$), and x is the measured osmolality.

Table 1. Values of Osmolality of the Different Milk Samples and the Corresponding Freezing Point Values

Sample	Osmolality/(mmol/kg)			Mean T_f/°C
	Reading 1	Reading 2	Reading 3	
Whole milk	278	275	277	–0.515
Whole milk + 5% water	262	261	263	–0.487
Whole milk + 10% water	251	247	249	–0.463
Fermented whole milk	345	343	343	–0.639
Skim milk	286	284	287	–0.531

Experimental Procedure

Part I: Quality Test of Milk

The following samples are prepared and analyzed: (i) whole milk; (ii) whole milk adulterated with the addition of 5% in volume of water; (iii) whole milk adulterated with the addition of 10% in volume of water; (iv) fermented whole milk, obtained by leaving fresh pasteurized milk for at least two days at room temperature to undergo lactic fermentation; and (v) skim milk. The osmolality values of each sample are measured with a Fiske osmometer, repeating typically three readings with different aliquots.

Part II: Determination of the Effective Molar Mass of Milk

Powdered milk is used as substance of unknown molecular weight. Both whole or skim powdered milk can be used, although the latter is easier to dissolve. Solutions are prepared by dissolving different quantities of powdered milk in a certain volume of water. Typical quantities are between 0.010 and 0.100 gram powdered milk per milliliter (gram) water. The solutions are homogenized by shaking or stirring for several minutes. Then, osmolality measurements are performed, repeating at least three readings with different aliquots.

Hazards

There are no significant hazards involved in this experiment. Nevertheless, the students should be advised not to drink from the milk samples owing to the risk of contamination.

Results and Discussion

Part I: Quality Test of Milk

Student results are shown in Table 1. For each sample, the values of osmolality obtained in the three repeats are in good agreement, with a standard deviation of less than 1%. From these values, the freezing points of the samples are calculated.

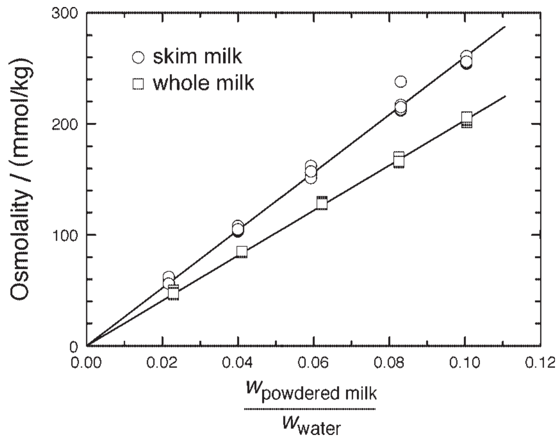

Figure 1. Osmolality values of solutions of different quantities of powdered milk in water. Note that the osmolality values are directly proportional to ΔT_f (eq 2).

The value obtained for the freezing point (T_f) of whole milk is perfectly in agreement with the legal standard given for cow milk from our region (Galicia, Spain): $T_f = -0.526 \pm 0.017$ °C (uncertainty indicated as 3 σ). When comparing with the freezing point of skim milk, slightly different values are obtained but both of them are within the legal range. This result may be surprising at first, but one has to consider that only the milk components in solution contribute to the freezing point depression, so that fat particles do not have any effect on it *(1, 4)*.

The data in Table 1 show the sensitivity of the freezing point depression to adulteration by addition of water, even for small quantities as those used in the experiment. Addition of water causes a decrease of osmolality that is significantly larger than the observed uncertainty in this quantity. Moreover, the decrease in osmolality with respect to untreated milk correlates linearly with the quantity of water added, as expected from the linear relation between freezing point depression and solute molal concentration in diluted solutions

$$\Delta T_f = T_f - T_f^* = -K_f\, m_B \tag{2}$$

where K_f is the cryoscopic constant of the solvent (in the case of water $K_f = 1.86$ °C kg mol^{-1}) and m_B is the molality of the solution. Addition of water causes a decrease of solute concentration and leads to a less negative freezing point of the solution.

Finally, the effect of lactic fermentation on the freezing point of milk is dramatic (Table 1). An increase of osmolality is observed of about 15–25%, depending on the fermentation stage of the sample. This means that the freezing point of milk decreases owing to lactic fermentation. This can be easily understood since each molecule of lactose yields four molecules of lactic acid after fermentation, so that the number of particles in solution increases significantly with this process. This is a good example of the “colligative” nature of freezing point depression.

Part II: Determination of the Effective Molar Mass of Milk

The plots of osmolality versus weight ratio between powdered milk and water for solutions of whole and skim milk are shown in Figure 1. Linear variations are observed for both types of milk, as expected from the relation between freezing point depression and weight ratio between solute and solvent derived from eq 2,

$$\Delta T_f = -\frac{K_f}{M_B}\frac{w_B}{w_A} \tag{3}$$

where M_B is the molar mass of the solute, w_B the weight of solute, and w_A the weight of solvent. Using this relation, the molar mass of the solute is obtained from the slopes of the lines in Figure 1. The values for whole milk and skim milk are given in Table 2.

Table 2. Effective Molar Mass of Whole and Skim Milk Obtained Using Different Methods

Type of Milk	M_B/(kg mol^{-1})	
	From Slopes in Figure 1	Extrapolation to Zero in Figure 2
Whole	0.492 ± 0.001	0.478 ± 0.004
Skim	0.384 ± 0.002	0.376 ± 0.006

NOTE: Uncertainties determined from linear regression and given as one standard deviation.

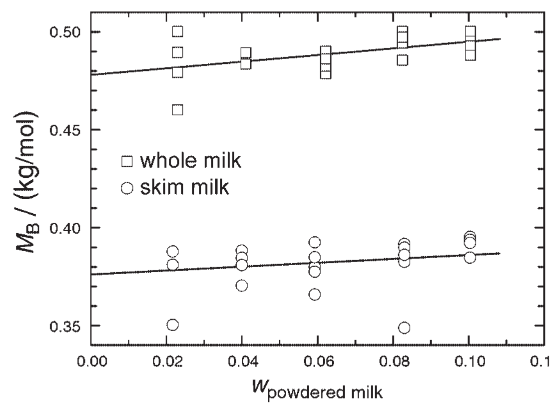

Figure 2. Plot of the effective molar mass calculated for the data in Figure 1.

It is well known that eq 3 is only valid for dilute solutions and must be refined by including higher-order terms for concentrated solutions *(3)*. Therefore, a more accurate determination of the molar mass can be achieved by extrapolation to zero solute concentration in the plot of the solute molar mass values calculated for each solution versus the corresponding weight ratios solute–solvent. M_B is obtained from the experimental data using the relation given by eq 3 reordered as follows:

$$M_B = -\frac{K_f}{\Delta T_f}\frac{w_B}{w_A} \tag{3}$$

Figure 2 shows the plots of the calculated molar masses versus the weight ratios powdered milk/water corresponding to the data in Figure 1. Variation with solute concentration is small, but a clear decreasing tendency of the molar mass is observed as the milk concentration is decreased. Linear extrapolations lead to the values of molar masses given in

Table 2, which represent the best estimates from the experimental data.

At this point we have to think about the physical meaning of the molar mass obtained for milk. For a solution containing a mixture of solutes, M_B is the number-average molar mass of the solutes (i.e., the total weight of solutes divided by the number of moles of particles in solution). Nevertheless, if particles are present that do not dissolve but remain in suspension, as it is the case of milk, this average molar mass is just an effective molar mass, since these particles have no effect on the freezing point depression but contribute to the total weight of powdered milk. Therefore, the effective molar mass of a mixture depends very much on the presence of suspended particles. This can be seen in the effective molar masses obtained above (Table 2). The effective molar mass of whole milk is significantly larger (about 21%) than that of skim milk. The difference can be explained on the basis of the fat content of the two types of milk, which contributes to the total mass of solute but not to the number of particles in solution.

Acknowledgments

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[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

Literature Cited

1. Mitchell, G. E. *Aust. J. Dairy Technol.* **1989,** *Nov,* 61–64.
2. Halpern, A. M.; Mcbane, G. C. *Experimental Physical Chemistry: A Laboratory Textbook,* 3rd ed.; W. H. Freeman & Company: New York, 2006.
3. Garland, C. W.; Nibler, J. W.; Shoemaker, D. P. *Experiments in Physical Chemistry,* 7th ed.; McGraw-Hill: New York, 2002.
4. Rohm, H. *Z. Lebensm. Unters. Forsch.* **1993,** *197,* 558–561.