

The Physical Chemistry, Theory and Technique of Freezing Point Determinations



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1 Physical Chemistry Review

1.1 Measuring the concentration of solutions

1. Definition of a solution: A homogeneous single-phase mixture of solute and solvent, where:
 - a. **Solvent** = the liquid component.
 - b. **Solute** = the solid component, or liquid in lesser proportion.

Suspensions or colloids are not solutions. The solvent must fully dissolve the solute.

2. Two common expressions of concentration:
 - a. **Molarity** = grams of solute per liter of solution.
 - b. **Molality** = grams of solute per kilogram of solvent.

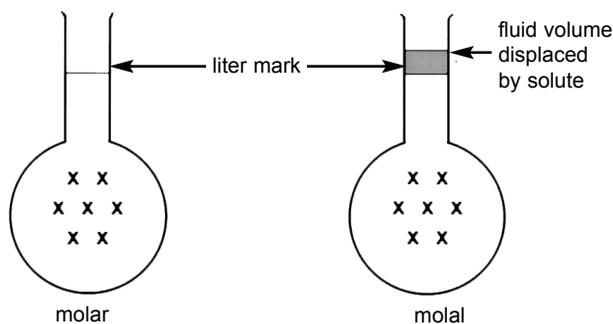


Figure 1: A 1 molal solution is generally more dilute than a 1 molar solution.

3. Methods of molality measurement... definition of osmolality

a. Colligative properties:

When one mole of non-ionic solute is added to one kilogram of water, the colligative properties are changed in the following manner and proportion:

- freezing point goes down 1.86°C
- osmotic pressure goes up 17000mmHg (17000mmHg = 22.4 atmospheres)
- boiling point goes up 0.52°C
- vapor pressure goes down 0.3mmHg (vapor pressure of pure water = 17mmHg)

b. Osmolality:

- One mole of non-ionic solute dissolved in a kilogram of water will yield Avogadro's number (6.02×10^{23}) of molecules.
- One mole of ionic solute (e.g., NaCl) dissolved in a kilogram of water will yield almost twice as many particles. The colligative properties are changed almost twice as much.
- Osmolality = $\phi \times n \times \text{molality}$
 - ϕ = osmotic coefficient, or percent deviation from "ideal" behavior (complete dissociation, no water binding, etc.).
 - n = number of particles into which the molecule can be broken (1 for sucrose, 2 for NaCl, 3 for K_2SO_4 , etc.).
- One osmol of any solute plus one kilogram of water will yield Avogadro's number of particles.
- Avogadro's number of particles, regardless of their size or shape, will change the colligative properties the amount described in 3a. (when dissolved in a kilogram of water).

c. Converting other units to milliosmoles (mOsm/kg):

mEq/l.....	divide by wc multiply by ϕ divide by v
ppm.....	divide by wc multiply by ϕ divide by v divide by atomic weight (aw)
mg%.....	divide by wc multiply by ϕ divide by mw multiply by n multiply by 10
millimolarity.....	divide by wc multiply by ϕ multiply by n
millimolality.....	multiply by ϕ multiply by n
millidegrees C.....	divide by 1.86

Explanation of symbols:

wc = percent water content of solution, or, (wet weight - dried-weight) / wet weight
 ϕ = osmotic coefficient
v = valence
aw = atomic weight
mw = molecular weight
n = number of particles into which the molecule can be broken.

A few examples of converting some common concentrations into milliosmol values follow.

Example 1:

What is the osmolality of a 4% solution of formaldehyde?

$$mw = 30.3$$

$$\text{formula} = \text{CH}_2\text{O}$$

$$n = 1$$

Assume $\phi = 1$

Assume wc error is negligible

Therefore: 30 gms formaldehyde + 1000 gms H₂O = 1000 mOsm

A 4% solution = 40 gms formaldehyde + 1000 gms H₂O

$$\text{Osmolality} = (40/30) \times 1000 = 1333 \text{ mOsm}$$

If you know:	and you measure:
the normal or original freezing point of a solution and that contamination has taken place	The freezing point of the solution
The weight of Solute A and the freezing point of Solvent B	The freezing point of A plus B
The specific gravity of a solution	The freezing point of the solution
The freezing point of a solution at time A	The freezing point of solution at time B
The type of dissolved molecule, the weight of solute, and the weight of solvent	The freezing point of the solution
The molecular weight of the dissolved solute molecule, the weight of solute, and the weight of solvent	The freezing point of the solution

Example 2:

What is the osmolality due to urea of a serum with a blood urea nitrogen (BUN) level of 10 mg%?

mw of urea = 60.06

mw of BUN = 28

Formula of urea = H_2NCONH_2

$n = 1$

Assume $\phi = 1$

Assume wc error is negligible

Therefore: 28 gms BUN + 1000 gms H_2O = 1000 mOsm

10 mg% = 100 mg + 1000 gms H_2O

$(100 \text{ mg}/28 \text{ gms}) \times 1000 = 3.6 \text{ mOsm}$

you can determine:	the application is:
The percentage dilution or change in concentration	Concentration of body fluids or quality control of standards and solutions
The volume of the container occupied by B	Measuring volumes of containers, tanks, kettles, pipets, human body, etc
The approximate size and number of dissolved molecules	Measuring the dialyzability of solute.
The change in number of molecules due to diastasis or other factors. If the freezing point is measured over a number known time intervals, then the rate of reaction can be measured	Measurement of enzyme activity
The molecular weight of the solute	Molecular weight determinations
The osmotic coefficient of the solute in the solvent	Determination of water binding, activity, etc.

Example 3:

What is the osmolality of a 0.15% ethanol solution?

$$\text{mw} = 46$$

$$\text{formula} = \text{C}_2\text{H}_5\text{OH}$$

$$n = 1$$

$$\text{Assume } \phi = 1$$

Therefore: 46 gms ethanol + 1000 gms H₂O = 1000 mOsm

1% = 10 gms ethanol + 1000 gms H₂O = $(10/46) \times 1000 = 218$ mOsm

0.15% = 1.5 gms + 1000 gms H₂O = $(1.5/46) \times 1000 = 32$ mOsm

d. Comparison of techniques.

Any of the colligative properties may be used to indicate osmolality. There are advantages and disadvantages to each one.

1. *Vapor pressure.* This technique allows the measurement to be made at the temperature desired. It is more complicated, however, not as reproducible in the biological range, and the instrumentation is more expensive. Some vapor pressure osmometers will not detect volatile solutes, which compromises their application in the clinical laboratory.
2. *Osmotic pressure.* Although this method is the most desirable from several points of view, it is difficult to obtain "ideal" membranes, especially for biological solutions. The primary use of "membrane" osmometers is in determining the molecular weights of large molecules. These instruments are very expensive.
3. *Boiling point.* Ebulliometry has been used extensively for the measurement of molecular weights. It is a relatively simple and inexpensive technique. The major limitation, especially with biological fluids is the destruction of the solute molecules at the boiling temperature.

4. *Freezing point.* With the available commercial instruments, freezing point is the most sensitive of the colligative techniques for the biological range of concentrations. In addition, it is the only technique in which the sample may be recovered. The table on the preceding pages represents the range of application for freezing point osmometers.

1.2 Comparison of concentrative indices

- General. There are many other methods of measuring the concentration of solids in liquids. Many of these, however, include the effect of non-solutes (undissolved particles) as well as solutes.
- Six non-specific concentrative indices and their dependence upon certain physical parameters of the solids:

Commonly used non-specific concentrative indices	Physical parameters of solutes and solids					
	number of particles	size of particles	shape of particles	charge	undissolved particles, number size, shape	opacity
Specific Gravity (density)	1	1	1	2	1	
Refractive Index	1	1	1	2		
Conductivity	1,2	2	2	1	1,2	
Viscosity	1	1	1	2	1	
Turbidity	1	1	1		1	1
Freezing Point	1					

“1” indicates primary dependence. “2” indicates secondary dependence.

Freezing point (osmotic concentration) depends only on the number dissolved particles, not on their size or shape.

Refractive index, which does depend on the size and shape of the dissolve molecules, is most closely related to the total dissolved solids.

3. Freezing point and specific gravity comparison:

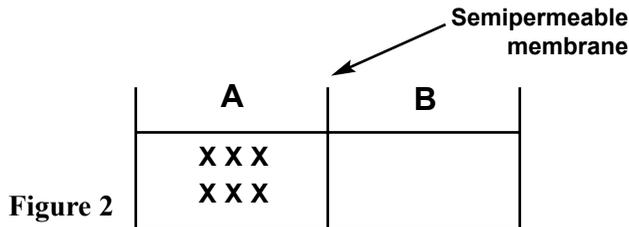
mOsm	solute	specific gravity
500	NaCl	1.006
500	Sucrose	1.029

The freezing point combined with specific gravity or refractive index can be used to indicate the approximate size of the dissolved solute (for instance in urine). It can be a very accurate indicator of molecular weight, as well as a means for monitoring changes in molecular weight as in hydrolysis or other chemical and enzymatic reactions.

1.3 Osmotic concentration and fluid movement.

1. Osmotic concentration (osmolality) is the concentration of osmotically active particles.
2. Total osmotic pressure. Osmotic pressure is the tendency for solvent to pass through a membrane from a solution of lower concentration to a solution of higher concentration.

Total osmotic pressure is an "ideal" concept. To define it, it is necessary to construct a model with an "ideal" semipermeable membrane as well as "ideal" solute and solvent. In this "ideal" system, the solvent will pass freely through the membrane, while the solute will not pass. In Figure 2, the total osmotic pressure is the force necessary to prevent water transfer from B to A.



If A and B differ in concentration by one milliosmol, the osmotic pressure will be 17mmHg.

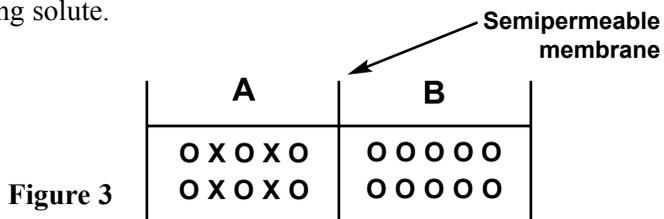
3. Effective osmotic pressure or tonicity.

Most membranes will allow some solutes to pass through as well as solvent.

The effective osmotic pressure, or tonicity, is the force exerted as a result of the solute(s) that does not pass through.

In Figure 3, the osmotic pressure can be expressed as a difference between the osmotic concentration (osmolality) of A and the osmotic concentration (osmolality) of B.

This difference, of course, is simply the concentration of the non-diffusing solute.

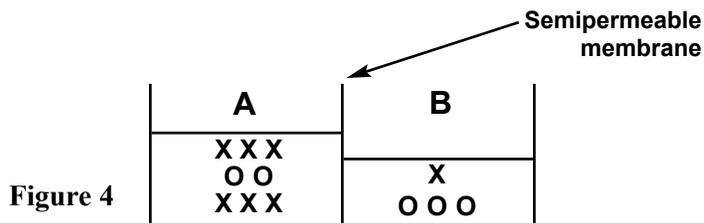


4. Fugacity or water potential.

Fluid movement is the resultant of many forces, only one of which is osmotic pressure. Hydrostatic pressure and electromotive forces are perhaps the other two most important.

This resultant, or net, force is called fugacity or water potential.

In Figure 4, if water moves from compartment A to compartment B, for any reason, then A is hyperfugacic to B.



5. Diffusion rates.

In addition to being "semi-permeable" (allowing some solutes to pass and preventing others), most membranes will allow some solutes to pass through faster than others.

This variation of diffusion rates means that two solutions which are iso-osmotic (which have the same osmotic concentration or osmolality) are not necessarily iso-tonic.

Consider our two compartments, A and B, again. Each contains 500 mOsm of solute, but A contains one kind and B contains another.

The solute in A may diffuse into B, but the solute in B may not diffuse into A. Or, the solute in A may diffuse faster than the solute in B. In either case, there will be at least *some* time during which the two solutions will *not* be iso-tonic.

6. Importance of "the system".

As opposed to osmotic concentration, osmotic pressure, tonicity and fugacity are all *relative* concepts, e.g., a solution does not have an osmotic pressure unless there is a semipermeable membrane present and another solution on the other side.

When talking about these terms, then, reference must be made to the particular system being studied.

2 Freezing Point Theory & Technique

2.1 Definitions

1. **Freezing Point** is the temperature at which a solvent or solution will turn from liquid to solid. More accurately, it is the temperature at which an infinitesimal amount of the solid phase will exist in equilibrium with the liquid phase.
2. **Melting Point** is the same temperature as the freezing point of a solvent or solution only the measuring technique is run in reverse.
3. **Supercooling** is the tendency of any solvent or solution to cool below the freezing point in a liquid state. The greater the amount of supercooling, the less stable the liquid and the more likely it is to crystallize spontaneously. It corresponds, in a sense, to supersaturation of solute in a solvent.
4. **Heat of Fusion** is the heat that is released when a super-cooled liquid crystallizes. The process of crystallization considerably reduces the random molecular motion of the liquid state. This loss of molecular energy is released in the form of heat. This heat of "putting together" actually warms the solution causing the paradox of sample warming during freezing. For water, 80 calories of heat are released for every gram that turns from liquid to solid.
5. **Thermodynamics** is the study of heat transfer. With respect to the measurement of freezing point, it is an analysis of all the factors which effect heat flow to, from and within the sample.

2.2 Theory

1. Phase Diagram. Most matter can exist as either a gas, liquid or solid, usually called "phases" or sometimes "states". The factors which determine whether a substance will be in one phase or another are temperature and pressure.

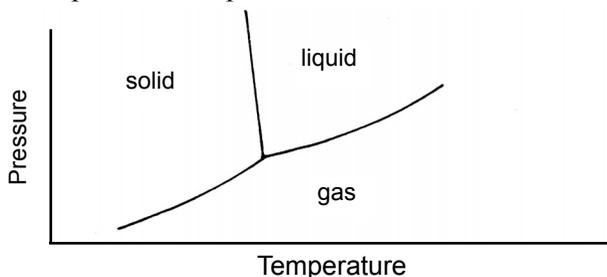


Figure 5: Phase Diagram for a Solvent

High pressures tend to liquify or solidify gases, while high temperatures tend to liquify solids, or gasify solids and liquids.

2. Addition of Solute. Adding solute to a solvent inhibits the tendency of the solvent molecules to escape into the gas phase, as well as inhibiting their ability to enter the solid phase.

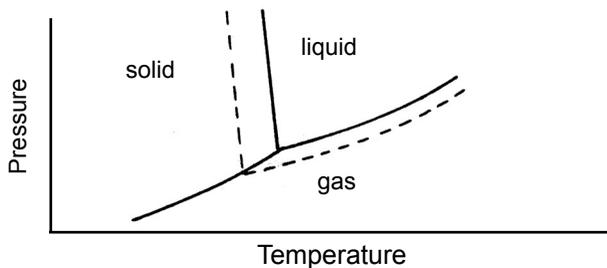


Figure 6: Phase Diagram with Addition of Solute

3. Freezing Point of a Solvent. When a pure solvent is cooled below its freezing point, one would ideally expect the following curve.

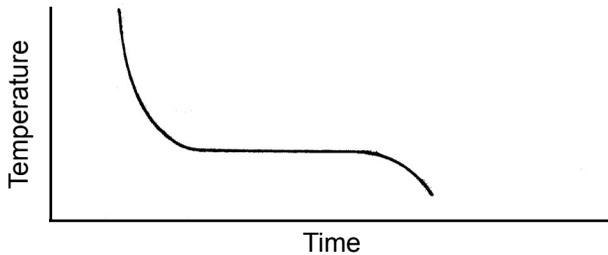


Figure 7: Solvent Freezing Curve "Ideal"

Initially the sample cools according to Newton's Law of Cooling (the rate of cooling is proportional to the difference in temperature between the sample and its environment). At the freezing point, the sample would remain constant in temperature until the entire mass had been converted to the solid phase. After that, the crystal mass would cool to the bath temperature.

Because all liquids have a tendency to supercool, however, the actual results one observes are as follows:

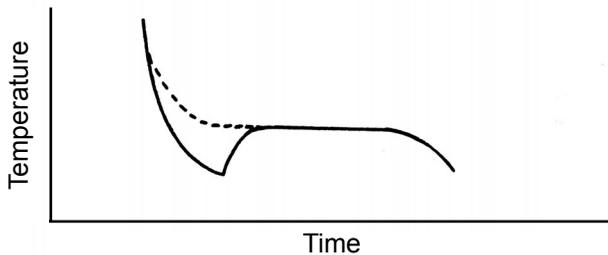


Figure 8: Solvent Freezing Curve with Supercooling

The sample cools below the freezing temperature until some impetus (seed crystal, vibration, etc.) starts crystallization. The temperature then rises to the freezing point and the process continues as in the "ideal" example.

3. Freezing Point of a Solution. For a mixture of solute and solvent, the situation differs. The solution does not freeze, only the solvent. The solute is solid already.

Theoretically, what one should observe as a sample is cooled past its freezing temperature is as follows:

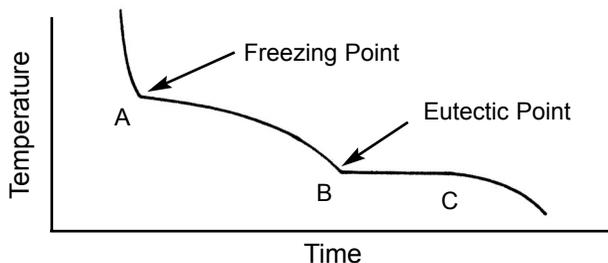


Figure 9: Solution Freezing Curve “Ideal”

Initially the solution cools the same way the solvent did. At this freezing temperature, "A", however, the solvent starts to crystallize out and the rate of cooling decreases sharply. This is because heat is added as the solvent crystallizes. As more and more solvent freezes out, the liquid portion of the solution becomes more concentrated. The increasing concentration lowers the equilibrium temperature between liquid and solid phases. Because there is only a fixed amount of solute, as more and more ice is formed, the concentration increases at an exponential rate. Finally the ratio of solvent in the liquid portion increases to the point where the solubility limit (eutectic point, "B"), is reached. As more solvent is frozen out, the solute precipitates out at a corresponding rate, which keeps the temperature constant. Finally, at "C", all the solvent becomes solid and the solid mass cools down to the bath temperature.

Because solutions also have a tendency to supercool, one would expect the actual curve to look similar to the following:

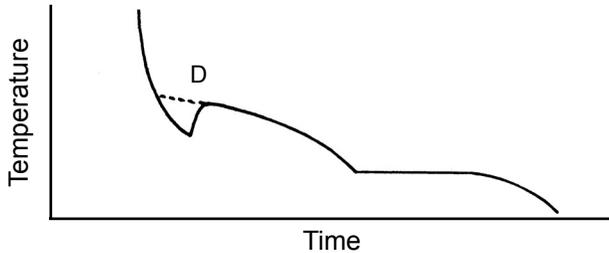


Figure 10: Solution Freezing Curve with Supercooling

In other words, one would expect the sample to cool below the freezing point, crystallize, then warm up and return to the "ideal" curve at point "D". In a cryoscope this does not happen, however, because of the manner in which the sample temperature is being measured.

What one actually sees is a curve like the following:

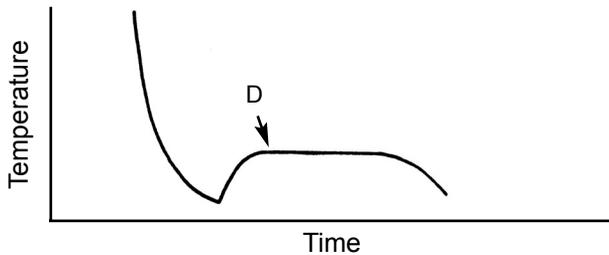


Figure 11: Solution Freezing Curve in a Cryoscope

After crystallization occurs the sample warms up to point "D" on the curve. Instead of following the curve shape of Figure 9, the sample temperature remains constant. It does so because the ice crystals form a "blanket" of slush which insulates the center of the sample from the cold bath.

The center of the sample, thus becomes an "adiabatic" system independent of its environment. Any temperature changes that take place are due only to internal causes, i.e., the heat of fusion.

During this period of isolation the center of the sample alternates between thawing and freezing. The heat of fusion released when one crystal is formed, melts an adjacent one. The heat absorbed during this melting causes the first crystal to freeze again, etc.

This temperature plateau makes the measurement of the freezing point of a solution more easy to measure than would the "ideal" curve of Figure 4.

The slush blanket finally breaks down and allows the heat to leak out to the bath. The sample slowly turns solid and cools to the bath temperature.

The temperature plateau reached by the center of the sample is colder than the true freezing point of the solution. When the sample was supercooled and then crystallized, some of the solvent turned to ice immediately. The remaining liquid solution is thus concentrated according to the amount of solvent removed (this can be calculated . . . the degrees of supercooling divided by the heat of fusion).

The supercooling error can be corrected for by using standards which "bracket" the unknowns, and by supercooling standards and unknowns the same amount.

2.3 Thermodynamic Factors

There are many factors which affect the rate of heat flow from the sample to the bath. These factors affect the shape of the freezing curve.

- a. Self-heating in probe - thermistor cryoscopes employ a probe through which current is passed. The resistance varies with temperature. Because the measuring current also heats the probe, it is important to use as little current as possible.
- b. Heat capacity of probe - the specific heat of the probe affects its response time. This is a critical factor in determining the smallest volume that can be measured. Generally, the smaller the probe, the smaller the sample that can be measured. A mercury thermometer has a relatively high specific heat and, as a result, is slow in responding to temperature changes.

- c. Isolation of probe - the degree to which the probe tip is thermally isolated from its stem determines how much heat will leak into the sample.
- d. Isolation of stir wire - the same isolation factors apply to the stir wire. The smaller it is in diameter, the less heat will leak in. Microsample instruments have eliminated the stir wire completely. Freezing is induced by an external vibration.
- e. Control of bath temperature - large fluctuations in bath temperature will lower the reproducibility, especially in small samples. Cold blocks provide excellent temperature control while eliminating the maintenance requirement of a liquid bath.
- f. Sample tube size and thickness - uniform dimensions in shape and thickness determine the heat flow pattern from the sample to the bath. Symmetry and uniformity are especially important in small samples. Injection-molded plastics have improved sample handling characteristics, while providing the convenience of a low-cost disposable product.

2.4 Conclusion

Today's modern cryoscope has incorporated these design factors into a simple and reproducible instrument, based upon the uniform freezing curve of Figure 11. The level of automation designed into today's cryoscope has eliminated all the variables of routine operation, and most routine maintenance as well. Over time, however, it is probable that even the most robust instrumentation will have at least some service requirement. It is for this reason that we provide a review of chemistry and thermodynamics. This is not meant to take the place of your instrument manual, which should in all cases be your primary guide to instrument operation and service. We hope that by providing this background we can enhance your appreciation of the technique of freezing point measurement, and make the results even more useful to you.