Phase Diagrams

Mixtures of Phases of Matter

Phase diagrams are used to describe equilibrium situations in which two or more phases of matter exist together in pure substances or in solutions. They are widely used in the physical sciences, especially in the fields of metallurgy, materials science, geology, and physical chemistry. In these fields, substances are often formed at high temperatures and then subsequently cooled to the solid state. The manner in which they are cooled determines the mixture of phases that exists when they become solid. This can have an enormous impact on the physical properties of the solid material due to internal stresses (e.g. tempered steel).

Phase diagrams have seen very little use in biology, however they have been widely appreciated in cryobiology since Cocks and Brower published an article showing their utility (*Cryobiology* **11**: 340-358, 1974). In biological systems, the primary component is water; the entire system is a collection of compartments filled with an aqueous solution. As aqueous solutions are cooled, the water forms a crystalline solid (ice) which has almost no solubility for the solutes that were in the aqueous solution. As ice forms, then, the solutes will be confined to the remaining liquid phase, becoming more concentrated. Since this lowers the freezing point of the aqueous liquid, the system can remain in equilibrium with a substantial unfrozen fraction. As cooling continues, the solubility limit of the solution will also be reached, leading to the precipitation of solutes. These events are succinctly described by a phase diagram.

Binary Phase Diagrams

The simplest type of phase diagram is for binary systems; systems in which there are only two phases present. The following diagram shows the phase diagram for sodium chloride and water, the most important solution for physiological systems.

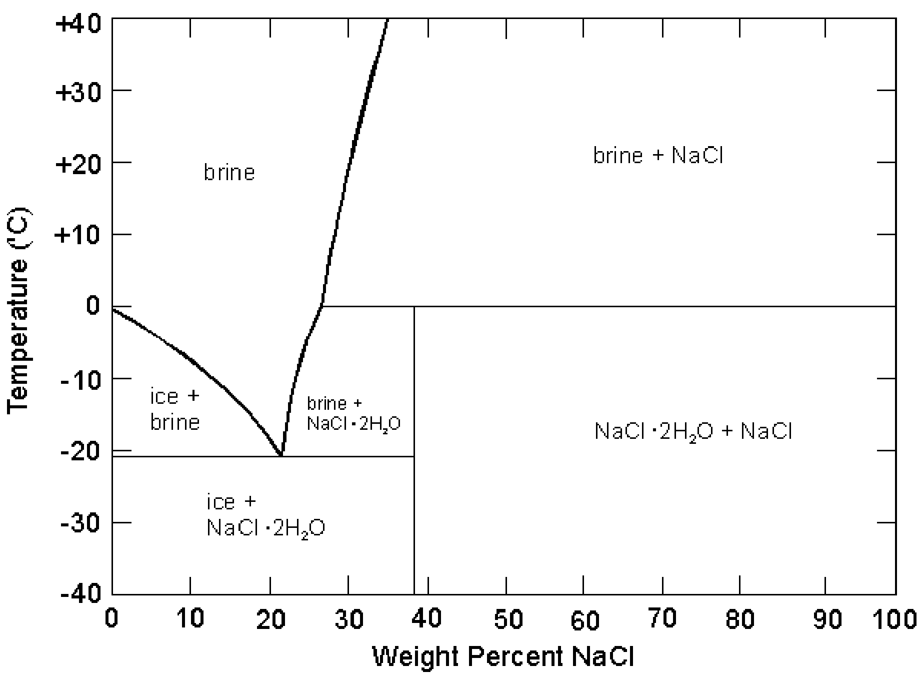

Fig. 6.1.1

Starting at the left hand side of the diagram, if the temperature of a solution with 0% salt is lowered, the freezing point occurs at 0ºC. If the solution has salt dissolved in it (i.e. the concentration of salt is below the solubility limit), then the mixture will exist in the brine compartment. As the temperature is lowered, the weight percent of NaCl doesn't change until the thick line is reached. This line defines the freezing point of the solution. Further cooling will take the solution along the curve defined by the thick line until the eutectic point is reaches at -21.2ºC. At this point, the unfrozen compartment of the mixture is saturated with NaCl; any further cooling will cause salt to precipitate out of the mixture.

For freezing biological systems, this left side of the phase diagram is the most important as it describes the osmolality of the solution in which the cells exist. For convenience, this curve can be described by a simple quadratic equation:

$$\pi = -0.52823T - 0.00492T^2 \qquad (6.1.1)$$

The osmolality will follow this curve down to -21.2ºC, where it will hold constant until the temperature is raised once again. Later on we will see how Jim Lovelock applied this phase diagram to cryobiology by explaining the freezing injury suffered by red blood cells, as well as providing an explanation of the cryopreservative effects of such compounds as glycerol and DMSO.

Ternary Phase Diagrams

Although the binary phase diagram for sodium chloride in water is useful for understanding injury to cells, most cryopreservation protocols use at least one cryoprotective additive to reduce the freeze-thaw injury. In such cases, there are three compounds that must be considered: water, NaCl, and the cryoprotectant. A system with three components is described by a ternary phase diagram. These are difficult to draw on paper since three dimensions are required to adequately represent the surfaces between phase boundaries. What is often done is to hold the ratio of solutes constant for a particular two dimensional figure, and plot the phase boundaries for that mixture. Diagrams of this nature have been published for the ternary systems DMSO-NaCl-H_2O and glycerol-NaCl-H_2O. From these diagrams, it is clear that the solubility and eutectic behaviour of a single solute can be altered significantly by the amount and type of additional solutes introduced into the system (e.g. DMSO serves as a solvent for NaCl as well as being a solute in water). It is also clear that equilibrium between solids and liquid becomes increasingly complex as the number of components is increased.

Glycerol-NaCl-Water

The ternary system glycerol-NaCl-H_2O can be described (Pegg, *Cryo-Letters* **4**: 259-268, 1983) using the following equation to obtain the melting point of a given solution (an inverse function will give the composition of the solution for a given temperature):

$$T_m = \left[-1.6 - 1.27R - 0.25R^2\right]^{-1} C - 0.01C^2 \qquad (6.1.2)$$

Where *R* is the weight ratio of glycerol:NaCl and C is the total concentration of solute (g/100g). This equation (and its inverse) will hold as long as the system is above the eutectic, described by the following equation:

$$T_e = -21.1 - 4.55R - 0.55R^2 \qquad (6.1.3)$$

DMSO-NaCl-Water

The ternary system DMSO-NaCl-H_2O can be described (Pegg, *Cryo-Letters* **7**: 387-394, 1986) using the following equation to obtain the melting point of a given solution (an inverse function will give the composition of the solution for a given temperature):

$$T_m = \left[-0.6 + 0.17\tan^{-1}(R)\right]C + \left[\tan^{-1}\left(\frac{R}{2}\right)/132 - 0.001\right]C^2 - 0.00045C^3 \qquad (6.1.4)$$

Where *R* is the weight ratio of glycerol:NaCl and C is the total concentration of solute. This equation (and its inverse) will hold as long as the system is above the eutectic, described by the following equations:

When 0 <= R

$$T_e = -21.2 - 6.155R + 0.157R^2 \qquad (6.1.5)$$

When 3 <= R

$$T_e = -150.3 - 63R - 8.55R^2 \qquad (6.1.6)$$

When 4 <= R

$$T_e = 37.29 - 26.42R + 2.466R^2 - 0.1028R^3 + 1.608\cdot 10^{-3}R^4 \qquad (6.1.7)$$

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