A Lecture on Colligative Properties in an Undergraduate Curriculum

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INTRODUCTION

Basic Pharmaceutics at the University of North Carolina is taught as a two-semester sequence (Phar 52 and Phar 53) during the third year of the five-year BS Pharmacy curriculum. These two courses are three credit hours each with one additional credit hour for the lab components of these courses. Although pharmacy as other health professions is changing almost constantly, there are many physical and chemical concepts that represent the foundation of our profession and these change little in their significance even though their application may change.

One topic that I emphasize and enjoy teaching and discussing with the students is the colligative properties of pharmaceutical solutions and their importance in everyday practice. This topic is one of my favorite lectures.

Since the significance of colligative properties is indisputable in the parenteral, ophthalmic and nasal solutions, it is necessary to introduce the students to these concepts as part of their pharmacy education. The purpose is to provide students knowledge to avoid problems arising from erroneously compounded (and at times wrongly dispensed) formulations where little attention was paid to their tonicity. In the Basic Pharmaceutics I sequence (Phar 52) two 50-minute lectures are dedicated to the explanation of principles of colligative properties using many examples of how to calculate the adjustment of tonicity of pharmaceutical solutions through colligative properties. The students are given extensive homework with numerous examples of problems involving calculations.

Although our students begin their pharmacy education with five prerequisite chemistry courses, they are exposed only briefly to the definitions and concepts of colligative properties. Very seldom are they aware of the application, and usefulness of these properties in pharmacy practice.

These first-year professional students seem very fascinated and interested in all the topics related to the colligative properties of pharmaceutical solutions and they have performed extremely well in solving problems in written examinations. At the beginning of the lectures, the students are given a handout with a brief description of the topics that will be used for explanation (Appendix). Many figures and tables are used in the classroom presentations to graphically demonstrate and explain some of the basic concepts of colligative properties. Due to space restriction these are not included in this manuscript. The following text is the summary of two lectures on colligative properties.

COLLIGATIVE PROPERTIES

Definition. Those properties that depend on the number of particles (molecules or ions) of the solute rather than on their physical and chemical properties are called colligative properties.

Description. The colligative properties of solutions are: (*i*) vapor pressure lowering; (*ii*) boiling point elevation; (*iii*) freezing point depression; and (*iv*) osmotic pressure.

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Osmotic pressure is the most important of the colligative properties since it is related to the physiological compatibility of parenteral, ophthalmic and nasal solutions. It is difficult and inconvenient to measure it and therefore other colligative properties (particularly freezing point depression) are determined and are then related to osmotic pressure. This is possible because all colligative properties are interrelated.

Vapor Pressure Lowering. The pressure brought by the vapor in equilibrium with its liquid is called the vapor pressure. It increases upon increasing the temperature. The vapor pressure of a pure liquid depends on the rate of escape of the molecules from the surface. If the liquid is mixed with another substance, its concentration is decreased and the rate of escape is lowered(1).

In pharmaceutical solutions, the solute is usually nonvolatile and it does not contribute directly to the vapor pressure of the solution. However, its presence decreases the concentration of the solvent and its escape tendency, *i.e.*, the vapor pressure of the solution is lower than that of the pure solvent. The vapor pressure lowering is proportional to the number of solute molecular particles or ions. The effect of a solute on the vapor pressure may be determined in dilute solutions by applying the Raoult's Law (Eq. 1).

$$p_a = p_a^o x_a \qquad \text{(Eq.1)}$$

where: p_a is the partial vapor pressure of the solvent in the solution; $p_a°$ is the vapor pressure of the pure solvent; and x_a is the mole fraction of a.

Since $x_a + x_b = 1$ (x_b is the mole fraction of the solute), the above equation can be rewritten as

$$p_a = p_a^o (1 - x_b) \text{ or,}$$

$$\frac{p_a^o - p_a}{p_a^o} = x_b$$

showing that the relative vapor pressure lowering of the solution is equal to the mole fraction of the solute(2).

This concept can be applied to calculate the vapor pressure for an aerosol propellant since mixtures of liquefied gas propellents can be considered as solutions. Mixtures of propellants result in reduced concentrations of any one propellant in the surface, thus in a reduction in the rate of escape and vapor pressure lowering of each component.

Example:
In a two component aerosol propellant system (A and B), the partial vapor pressure of A is:

$$p_a = p_a^o x_a \text{ or } p_a = p_a^o \frac{n_a}{(n_a + n_b)};$$

the partial vapor pressure of B is:

$$p_b = p_b^o x_b \text{ or } p_b = p_b^o \frac{n_b}{(n_b + n_a)}$$

the total vapor pressure (P) is:

$$P = p_a + p_b$$

In a blend of two propellants (A/B = 30:70[g]) where pure propellant A (mol. wt 120.93) has vapor pressure of 84.9 psia and propellant B (mol. wt 137.38) has vapor pressure 13.4 psia respectively the partial vapor pressure for A is:

$$p_a = 84.9 \frac{\frac{30}{120.93}}{\frac{30}{120.93} + \frac{70}{137.38}} = 84.9 \frac{0.2481}{(0.2481 + 0.5095)} = 27.80 \text{ psia}$$

and

$$p_b = 13.4 \frac{\frac{70}{127.38}}{\frac{70}{127.38} + \frac{30}{120.93}} = 13.4 \frac{0.5095}{(0.2481 + 0.5095)} = 9.01 \text{ psia}$$

The total vapor pressure of propellants in the above aerosol then equals:

$P = p_a + p_b = 27.80 + 9.01 = 36.81$ psia or,

$P = 36.81 - 14.7 = 22.11$ psig

Applying the same principle of Raoult's law one can also calculate the volume of two propellants (*e.g.*, propane and isobutane) required to achieve a certain vapor pressure suitable for a propellant[2].

Boiling Point Elevation. The boiling point of a liquid is the temperature at which the vapor pressure of the liquid is equal to the external pressure of 760 mm Hg. Since the vapor pressure of a solvent is lowered when a nonvolatile solute is added, the result is that the solution must be heated to a higher temperature than the pure solvent to reach the same vapor pressure. The boiling point of a solution is thus elevated in comparison to the boiling point of the pure solvent.

By using the mathematical relation between vapor pressure and temperature and the Raoult's Law, an equation is derived for the boiling point elevation of a solution:

$$\Delta T_b = K_b m$$

where ΔT_b is the elevation of the boiling point, m is the molality of the solution and K_b is the proportionality constant, which can be also defined as the boiling point elevation for one molal (m) dilute solute solution *i.e.*, $K_b = \Delta T_b/m$. The values of K_b are different for different solvents; for water it is 0.515°C.(3)

From the above equation one can calculate the concentration of the solute in a solution by measuring the boiling point elevation and knowing the K_b.

Example:
Calculate the concentration of dextrose (mol. wt 180) in 1000 g of water if the boiling point elevation of the solution is 0.284°C:

$$\Delta T_b = K_b m \text{ or } 0.284 = 0.512 \text{ m}$$

then

m = 0.555 or 99.84 (100)g of dextrose in 1000 g of water.

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[2] Nash, R.A., from classnotes, St. John's University, Jamaica NY.

Freezing Point Depression. The freezing point of a liquid is the temperature at which the solid and the liquid phases are in equilibrium at one atmosphere. The freezing point of a solution is the temperature at which the solid phase of the solvent and the liquid phase of the solution are in equilibrium at one atmosphere.

By using the thermodynamic equations for the effect of the temperature on the vapor pressure of the solid and liquid phases and Raoult's Law, the following equation is obtained:

$$\Delta T_f = K_f m$$

where ΔT_f is the lowering of the freezing point of a solvent in a solution, m is the molality of the solute and K_f is the molal lowering of the freezing point. The K_f value for water is 1.858°C.

From ΔT_f equation the concentration of the solute in a solution can be calculated by measuring the freezing point depression of the solution and knowing the K_f of the solvent. The above two equations are valid only for *very dilute solutions.*

Example:
Calculate the concentration of dextrose (mol. wt 180) in 1000 g of water if the freezing point depression of the solution is 0.52°C.:

$$\Delta T_f = K_f m \text{ or } 0.52 = 1.86 \text{ m}$$

then

m = 0.280 or 50.4 (50)g of dextrose in 1000 g of water

Osmotic Pressure. Diffusion of a solvent through a semipermeable membrane from a less concentrated solution into a more concentrated solution is called the osmosis. The pressure that must be applied to the side of the concentrated solution to prevent the flow of the pure solvent into the solution is called the osmotic pressure of the solution. A semipermeable membrane is a membrane which allows the penetration of only the solvent molecules. If any of the solute diffuses through a membrane it is not a semipermeable membrane. Since the measurement of the osmotic pressure is difficult and the colligative properties are interconvertable, one can calculate the osmotic pressure from another colligative property as for example freezing point depression(4).

CLINICAL IMPORTANCE OF OSMOTIC EFFECTS

Osmotic pressure is important from a biological viewpoint since the physiological membranes (*e.g.*, red blood cell membranes) are semipermeable membranes. The effect of osmotic pressure on the red blood cells (RBC) can be demonstrated by suspending them in a solution of *e.g.*, five percent sodium chloride solution which is of greater osmotic pressure than their contents. The water in the RBC's will then pass through the semipermeable cell membranes into the saline solution *i.e.*, from conditions of lower to those of higher osmotic pressure. The cells will consequently experience loss of water, and will shrink and become wrinkled. The process is called *crenation.* Conversely, if the RBC's are suspended in a 0.2 percent sodium chloride solution (lower osmotic pressure), the water from the solution will penetrate through the cell walls into the cells causing them to swell, to increase in size and eventually to break, to release hemoglobin. This process is called *hemolysis*(4).

It is important, therefore, to make sure that the osmotic pressure of solutions for injection is about the same as that in the blood. Such solutions are called *isotonic* solutions. Solutions with a higher osmotic pressure are *hypertonic* and those of a lower osmotic pressure are said to be *hyptonoic.* Also, to reduce or avoid discomfort on administration of solutions to the delicate membranes (*e.g.*, ophthalmic, nasal, vaginal solutions) it is very important to ensure that the solutions are isotonic with the tissues.

A 0.9 percent solution of sodium chloride, or a five percent solution of dextrose, are iso-osmotic and isotonic with our body fluids. However, a 1.9 percent solution of boric acid although iso-osmotic with blood is not isotonic. The reason is that boric acid and some other agents (urea, ammonium chloride, alcohol, glycerin, etc.) will penetrate through RBC membranes because they are not truly semipermeable ones since they allow the penetration of some small molecules. However, boric acid and the other above mentioned agents give iso-osmotic and isotonic solutions for eye and nose treatment and can be freely used for adjustment of tonicity of solutions for this application.

NONELECTROLYTE VERSUS ELECTROLYTE SOLUTIONS

The osmotic pressure of solutions of different non-electrolytes is identical for identical molal concentrations. For example aqueous solutions containing 18.02 g of anhydrous dextrose, or 18.22 g of mannitol or 9.21 g of glycerin, in 1,000 g of water are all 0.1 molal and they have identical colligative properties. The reason is that they are nonelectrolytes and are thus not ionized when introduced into water and each molecule keeps its identity and remains as an entity. On the other hand, electrolytes will ionize and each ion will act as an entity. Therefore, equimolal solutions of electrolytes will have higher osmotic pressures (and all other colligative properties) than equimolal solutions of nonelectrolytes. A sodium chloride solution (NaCl) will have twice as many entities *i.e.*, Na^+ and Cl^-, a potassium sulfate solution (K_2SO_4) will have three times as many entities *i.e.*, $2K^+$ and SO_4^{2-} and a trisodium citrate solution ($C_6H_5O_7Na_3$) will have four times as many entities, *i.e.*,$3Na^+$ and $C_6H_5O_7^{3-}$ as a dextrose solution of equal molality. Therefore the freezing point depression of a solution of an electrolyte becomes:

$$\Delta T_f = iK_f m$$

where *i* is the van't Hoff's factor, *i.e.*, the number of ionic entities (for sodium chloride is 2, for potassium sulfate it is 3 and for trisodium citrate it is 4). Although at high dilutions, the *i* approaches the numbers of ions into which the electrolyte molecule ionizes, the value of *i* decreases as the concentration of the electrolyte solute increases. This is mainly because of ionic collision and interionic attraction which results in departure from the theoretical values for *i.* The latter can be determined by calculating the ratio of any colligative property measured for a molal solution to that of an ideal equimolal solution. Therefore, the molal freezing point depression in water of sodium chloride is 3.4 (instead of 3.72), of potassium sulfate is 4.2 (instead of 5.58), and of trisodium citrate it is 5.2 (instead of 7.44).

PREPARATIONS OF ISOTONIC SOLUTIONS

Calculating Isotonicity

Three methods are frequently used. Students make their own choice but are expected to know *two* of these methods. The reason is that only then can they check the correctness of their calculations and be sure of no error being introduced when preparing isotonic solutions. If carried out correctly, the three methods give closely comparable results with little deviation.

L-Method. In this method the freezing point depression equation is used to calculate the amount of the adjusting agent that must be added to bring to tonicity a hypotonic solution of a drug(5). Since the freezing point depressions for solutions of electrolytes are invariably greater than those calculated for the equation $T_f = K_f m$, a new constant L (= iK_f) is introduced to account for this deviation. The equation then becomes:

$$\Delta T_f = Lc$$

where L is the *molal* freezing point depression of water taking into consideration the ionization of the electrolytes, *i.e.*, iK_f. The value of *c* refers to the concentration of the solution expressed in molarity. In dilute solutions (as are the pharmaceutical solutions), the *molal* concentrations are not much different from the *molar* concentrations and are used interchangeably; in fact, the L values are tabulated in many textbooks as constants.

Example:
Calculate how many grams of sodium chloride (mol. wt 58.5) is required to make 250 ml of 0.5% w/v solution of lidocaine HCl (mol. wt 270.8) isotonic with blood. L_{iso} for both the drug and sodium chloride is 3.4. Using the equation $DT_f = L_{iso}c$, we calculate the freezing point lowering of the given solution:

$$\Delta T_f = 3.4 \frac{5}{270.80} = 0.063^{o}C.$$

i.e., the freezing point depression of the solution is 0.063°C.

Since the freezing point of body fluids is -0.52°C, and since the above drug in the concentration of 0.018 M/L reduces the freezing point by 0.063°C., the concentration of sodium chloride to be added to bring the solution to isotonicity, *i.e.*, to lower the freezing point by another 0.457°C. (0.52°C- 0.063°C) (using the same equation) is:

$$0.457 = 3.4\,c$$

and $$c = 0.457/3.4$$

i.e., $c = 0.134$ M/L

Thus the weight of sodium chloride to be added to make 250 ml of solution is:

$$0.134 \times 58.5 \times 250/1000 = 1.96 \text{ g}$$

Sodium Chloride Equivalent Method. This method uses the sodium chloride equivalent to calculate the amount of an adjusting agent needed to be added to a solution to bring it to isotonicity. The sodium chloride equivalent (E_{NaCl}) is the weight of the sodium chloride (in grams) that will produce the same colligative properties as one gram of a drug. For example, if the E_{NaCl} of a drug is 0.20 this means that 0.20 grams of sodium chloride will have identical osmotic pressure, freezing point depression (and other colligative properties) as one gram of the drug.

When using this method, the amount of the drug is multiplied by the E_{NaCl} to obtain the amount of sodium chloride that will produce identical osmotic conditions to those of the drug in the solution. This value is then subtracted from the amount of sodium chloride needed for an isotonic solution (0.9 g/100 ml). If the adjusting solute is not sodium chloride, the amount of calculated sodium chloride is divided by the E_{NaCl} of the adjusting solute. This then represents the weight of the adjusting agent to be added to bring the solution to isotonicity. The E_{NaCl} for a large number of drugs are tabulated in many textbooks (6).

Example (using the previous Lidocaine HC1 solution):
The E_{NaCl} of Lidocaine HCl is 0.22 and therefore the osmotic equivalent of 0.5 g Lidocaine HCl in the solution is:

$$0.5 \times 0.22 = 0.11 \text{ g sodium chloride}$$

This means that the 0.5 percent of Lidocaine HCl in the solution will have an osmotic pressure that is equivalent to 0.11 percent of sodium chloride in solution. Since an isotonic saline solution contains 0.9 g of sodium chloride in each 100 ml of solution, then the amount of sodium chloride needed to be added to the above solution to make it isotonic is:

$$0.9 - 0.11 = 0.79 \text{ g sodium chloride}$$

and for 250 ml it would need:

$$0.79 \times 250/100 = 1.975 \text{ g sodium chloride}$$

If dextrose was prescribed as the adjusting solute, then 1.975 would be divided by the E_{NaCl} of dextrose which is 0.16.

Cryoscopic Method. In this method the quantity of each substance required for an isotonic solution can be calculated from the freezing point depression values(2). As has been demonstrated, a solution which is isotonic with blood has a T_f of 0.52°C. and we must therefore adjust the freezing point of the drug solution to this value. Pharmaceutical textbooks usually list the freezing point depression of many compounds and it is then easy to calculate the concentration needed to achieve isotonicity from these values. For examples a one percent solution of sodium chloride has freezing point depression of 0.576°C. The concentration of sodium chloride required to make isotonic saline solution is then:

$$\frac{0.52}{0.576} \times 1.0 = 0.9\% \text{ w/v}$$

(where 0.52°C. is the freezing point depression of blood).

With a drug solution it is not possible to adjust tonicity by altering the drug concentration and an "adjusting" substance must be added to achieve tonicity.

The weight (in grams) of adjusting substance can be calculated. Taken that the drug concentration in 100 ml solution is x grams, then:

ΔT_f (for drug solution) =

x (ΔT_f of 1 percent drug solution) = a

Then, if w are the grams of the adjusting substance to be added to 100 ml of drug solution to make is isotonic:

ΔT_f (for adjusting solution) =

w (ΔT_f of 1 percent adjusting substance) = wb

For an isotonic solution:

$$a + (wb) = 0.52 \text{ or}$$

$$w = \frac{0.52 - a}{b}$$

Example (using the previous Lidocaine HCl solution): Calculate the amount of sodium chloride to be added to 250 ml of a 0.5% w/v Lidocaine HC1 solution to make it isotonic with blood.

From reference lists, the *b* for sodium chloride is 0.576°C. and for Lidocaine HCl it is 0.130°C, then:

$$w = \frac{0.52 - (0.52 \times 0.130)}{(0.576)} = \frac{0.52 - 0.065}{(0.576)} = 0.790 \text{ g}$$

Therefore the weight of sodium chloride to be added to 250 ml of solution is:

$$0.790 \frac{250}{100} = 1.97 \text{ g}$$

The above calculations demonstrate that any of the three methods can be used equally well to calculate the adjustment of tonicity of the pharmaceutical solutions.

SUMMARY

To avoid crenation or hemolysis of RBC's, to avoid pain and discomfort if solutions are injected or introduced into the eyes and nose, solutions should be made isotonic. This is true for either manufactured or extemporaneously prepared solutions. By using the appropriate calculations based on colligative properties of solutions, it is easy to determine the amount of adjusting agents to be added and in so doing to circumvent side effects from administering less or more than isotonic solutions.

The material that I cover in the two lectures is not new and can be found in standard pharmaceutical textbooks. However, I try to make the students aware of some of the physicochemical properties of the pharmaceutical formulations, to instill interest to the many aspects of pharmaceutics and to motivate them into thinking and understanding the course topics rather than simply accepting the facts, and then memorizing and remembering them mainly to pass examinations. In the majority of cases I succeed, and students show interest and enthusiasm for the lectures. I try to adjust my lectures to the mood/climate of the students using humor, open challenges, questions and discussions in the-attempt to make a difficult subject both exciting and interesting.

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(4) Hitchcock, D.I., *Physical Chemistry for Students of Biology and Medicine*, Little, Brown and Co. Boston MA (1953) pp. 41-44.
(5) *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Company, Easton PA (1995) pp. 1491 - 1497.
(6) Martin, A.N., *Physical Pharmacy*, 4th Ed., Lea & Febiger, Philadelphia PA (1993) pp. 129,137.

APPENDIX. LECTURESON COLLIGATIVE PROPERTIES

1. Read Chapter 5 of Martin's book (4th Ed.), pp. 110-122;
2. Solve the problems/calculations 5-14, 5-16, 5-18, 5-22, 5-25,5-27 and 5-28 at the end of the same Chapter (p. 123-124);
3. Solve the calculation assignments (1)-(9) from your handouts;
4. After Lectures 6-8 you will be able to:
   a. Define and understand the significance of colligative properties of solutions;
   b. Define "isotonicity", "hypertonicity" and "hypotonicity";
   c. Know and discuss differences between the colligative properties of nonelectrolyte-and electrolyte solutions;
   d. Apply equations for the specific colligative properties of drug solutions;
   e. Use three different methods to determine the tonicity of pharmaceutical ophthalmic and parenteral solutions and calculate the amount of adjusting agent to be used.

- Definition
  a. lowering of vapor pressure
  b. elevation of boiling point
  c. depression of freezing point
  d. osmotic pressure
- Application of Colligative Properties to Pharmaceutical Solutions
- Isotonic solutions have the same osmotic pressure as the body fluids (0.9% sodium chloride; 5% dextrose).
  Hypertonic solutions have osmotic pressure higher and hypotonic solutions have osmotic pressure lower than body fluids.
  Hypertonic solutions produce crenation of erythrocytes; hypotonic solutions produce hemolysis of erythrocytes
- Adjustment of Tonicity - Calculations
  a. L_{iso}-Method
  b. Sodium Chloride Equivalent (E_{NaCl}) Method
  c. Cryoscopic Method