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A Study of Isotonic Solutions*

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There is an increasing interest in the use of medicinal solutions having the same osmotic pressure as the body fluids or bearing a definite relation thereto. Originally the main interest in this field was concerned with parenteral solutions, but recently considerable attention has been given to adjustment of the osmotic pressure of collyria and nasal preparations.

Hypotonic solutions may be made isotonic by addition of more of the drug or some other substance. The proportion of substance to be added may be determined experimentally or calculated mathematically. In the present paper, the value, limitations and accuracy of these methods are discussed and new experimental data are presented.

THEORETICAL

The idea of isotony is connected with the knowledge of osmotic pressure, which was first observed by Nollet in 1748. The term "osmosis" was introduced by Dutrochet.

Plasmolytic Method.—In 1888, De Vries found that when certain vegetable cells were placed in solutions containing 7.5% or more of sucrose, water passed out from the cells, which then contracted away from the sheath of cellulose. This phenomenon is called plasmolysis. By varying the concentration of the solution it is possible to determine the concentration at which plasmolysis ceases or is barely detectable; such solutions are said to be isotonic with the cell sap, *i. e.*, capable of producing the same pressure in the cell. The abnormal osmotic pressure of salt solutions was first observed by De Vries, who introduced the term "isotonic coefficient" to express the degree of deviation from normal. The plasmolytic method is not trustworthy for accurate measurements, as it is subject to numerous errors, such as reaction of the cell contents with the substances studied, exosmosis of cell contents, etc.

Hemolytic Method.—The hemolytic method, which was developed in 1890 by Hamburger, consists in the determination of the concentration which produces laking of red blood corpuscles, which are semipermeable to solutions of most substances except urea and ammonium salts. Wokes (1) determined the ratio *isotonic concentration/hemolytic concentration*, and found that this ratio was different for different substances. Some of the ratios were as follows: sodium chloride, 2; dextrose, 2.9; sodium bicarbonate, 3.1. Boric acid was hemolytic in all concentrations. Wokes concluded that if a solution diluted with half its volume of distilled water is hemolytic, it is not isotonic and should not be used for injection.

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Osmotic Pressure Laws.—The experimental methods available for the study of osmotic pressure were improved in 1866 by Traube, who showed that animal and vegetable cells could be replaced by an artificial membrane of copper ferrocyanide. Studies by Pfeffer and van't Hoff established the following laws: (*a*) the osmotic pressure of a solution is proportional to its concentration, and (*b*) osmotic pressure is proportional to absolute temperature. These laws may be represented by the expression (I) $P = K(T/v)$, where P = osmotic pressure, K = a constant, T = absolute temperature and v = volume of solution. It was suggested by van't Hoff, and later verified by others, that the value of the constant in the osmotic pressure equation would have the same numerical value as the constant in the gas equation. The general gas equation is (II): $p = (n/v)RT$, in which p = pressure, v = volume in liters, n = number of gram-molecules, R = gas constant and T = absolute temperature. The corresponding expression for osmotic pressure is as follows (III): $P = (n/v)RT = wRT/Mv = cRT$, where P = osmotic pressure, v = volume of the solution in liters, M = molecular weight of solute, w = number of Gm. of solute and c = moles of solute per liter.

From equation (III), which holds for very dilute solutions of nonelectrolytes, it follows that solutions of the same molar concentration are isosmotic, *i. e.*, have the same osmotic pressure, provided the temperature is the same. The term "isotonic" is commonly considered as synonymous with "isosmotic," but the distinction is sometimes made that the osmotic pressure of a solution depends on the total molar concentration, whereas the tonicity depends on the molar concentration of those solutes which do not pass through the particular plasmatic membrane (21).

In direct relation with the osmotic pressure of a solution is its vapor pressure, which is lowered in proportion to the concentration of dissolved substance. Direct measurements of osmotic pressure and vapor pressure are difficult to carry out. Hence it is customary to make use of two phenomena which are directly proportional to the lowering of the vapor pressure, *i. e.*, the elevation of the boiling point and the lowering of the freezing point. As the freezing-point determination offers several advantages, it is commonly employed.

In 1788, Blagden found that lowering of the freezing point is proportional to the concentration of solute. Raoult, in 1882, showed that the freezing-point lowering is proportional to the molar concentration of the solute and that, for a given solvent, equimolar solutions of different nonelectrolytes have the same freezing point. The mathematical expression of Raoult's law is as follows (IV): $\Delta = K(1000\ w/ML)$, where Δ = freezing-point lowering of the solution as compared with the pure solvent, K = molar freezing-point lowering of the solvent (this is an individual constant for each solvent, based on the freezing-point lowering given by 1 mole of solute in 1000 Gm. of solvent), w = weight of solute, M = molecular weight of solute and L = weight of solvent plus solute.

Raoult's law is valid only when the pure solvent crystallizes from the solution on freezing. Likewise the law is a limit law which holds only for dilute solutions. In higher concentrations, deviations occur as is the case with the gas laws at higher pressures.

For dilute solutions of nonelectrolytes, Raoult's law may be used in making calculations for the preparation of isotonic solutions, as shown in the examples (2) which follow.

Example 1: Calculate the quantity of anhydrous dextrose to be used in preparing 100 Gm. of a solution isotonic with blood serum.

Transforming equation (IV), we have (V):

$$w = \Delta ML/K \times 1000$$

It is known that the freezing-point lowering of blood is 0.56° C. and that the molar freezing-point lowering of water is 1.86° C. Substituting these figures for Δ and K, respectively, replacing M by 180 (the molecular weight of anhydrous dextrose), and replacing L by 100 (weight of solvent plus solute), we have: $w = 0.56 \times 180 \times 100/1.86 \times 100 = 5.42$ Gm. Hence the solution should be prepared by dissolving 5.42 Gm. of anhydrous dextrose in 94.58 Gm. of water.

Example 2: Calculate the quantity of anhydrous dextrose to be used in a 1% solution of urea to make 100 Gm. of solution isotonic with the blood.

Using equation (IV), the freezing-point lowering caused by 1% of urea is calculated as follows: $\Delta = 1.86 \times 1.0 \times 1000/60 \times 100 = 0.31°$.

The freezing-point lowering caused by the urea is subtracted from the freezing point of blood, to determine the additional lowering to be brought about by the addition of dextrose, as follows: $0.56° - 0.31° = 0.25°$. Using equation (V), and making the required substitution, we have: $w = 0.25 \times 180 \times 100/1.86 \times 1000 = 2.42$ Gm. The solution should thus be prepared by dissolving 1.00 Gm. of urea and 2.42 Gm. of anhydrous dextrose in sufficient water to make 100 Gm.

Raoult's law cannot be used to calculate the concentration of isotonic solutions of electrolytes, since solutions of acids, bases and salts show an abnormally high osmotic pressure. For such compounds the isotonic coefficient increases with dilution. These facts were explained by Arrhenius in his theory of electrolytic dissociation, which assumes that in solution the molecules of electrolyte dissociate into ions and each ion has an effect on the osmotic pressure. Laws of equilibrium between ions and undissociated molecules have been established for weak electrolytes, but with strong electrolytes, discrepancies are observed. In modern theories it is assumed that strong electrolytes are fully dissociated in solution. Consequently the effect of dilution on

osmotic pressure is explained on the basis of an increase in ionic activity, rather than by an increase in the number of ions as suggested by Arrhenius. It is thought that the ions, because of their electric charge, influence each other in their free movement and that these effects are greater in more concentrated solutions. Nixon and Culbert (3) have shown that the assumption of complete dissociation may be accepted for practical work in the case of monovalent electrolytes, but that in the case of sulfates of divalent cations the same assumption leads to considerable error.

THE PREPARATION OF ISOTONIC SOLUTIONS

Nonelectrolytes.—The use of Raoult's law in calculating the concentration of solutions isotonic with the blood has already been shown (see examples 1 and 2). Calculations for solutions isotonic with the tear secretion can be made in the same way, with the exception that in the formula 0.56 is replaced by 0.80, since the tear secretion freezes at −0.80° C.

Electrolytes.—The ordinary form of Raoult's law cannot be applied to electrolytes, because it does not take into consideration the electrolytic dissociation or ionic activity of the electrolyte. For use with electrolytes, Raoult's law must be modified by inclusion of the factor i, which is the value of the isotonic coefficient. Equation (V) thus becomes (VI): $w = \Delta ML/K \times 1000\, i$. The value of i cannot be predicted exactly because it depends on the nature of the compound and the degree of dilution. For 0.9% NaCl solution, the value of i has been determined experimentally and found to be 1.86. This value indicates that the osmotic pressure of a 0.9% NaCl solution is 1.86 times as great as that of an equimolar solution of a nonelectrolyte. When dealing with electrolytes, the practical value of Raoult's law is thus dependent on the accuracy with which the value of i is determined or estimated.

Osmotic Factor Method.—The osmotic pressure of various solutions may be compared by calculating an "osmotic factor" for each solution (4). The osmotic factor is proportional to the number of particles in 100 cc. of the solution and is calculated as follows (*A*):

$$\text{Osmotic factor} = \frac{\left(\begin{array}{c}\text{Number of particles}\\ \text{from one molecule of}\\ \text{solute}\end{array}\right)\left(\begin{array}{c}\text{Gm. of solute}\\ \text{in 100 cc. of}\\ \text{solution}\end{array}\right)}{\text{Gram-molecular weight of solute}}$$

The problems which present themselves usually take the following form. A solution of definite percentage strength is prescribed with directions that it be made isotonic with the blood. The osmotic factor of the blood cannot be calculated directly, but its value may be taken as equal to that of an isotonic solution of sodium chloride. The concentration of sodium chloride solution which is isotonic with the blood is still a subject of discussion and values ranging from 0.7% to 1.0% have been used. The experimental values found by several authors vary from 0.9% to 0.96%. The U. S. P. physiological solution of sodium chloride containing 0.85% NaCl is slightly hypotonic. Assuming that average blood is isotonic with 0.90% NaCl solution, the calculations may be made on this basis: osmotic factor (for 0.90% NaCl solution) = $2 \times 0.9/58.5 = 0.031$. The next step is to calculate the osmotic factor of the drug prescribed, using formula (*A*). The osmotic factor of the substance to be added is then determined as follows (*B*): osmotic factor of substance to be added = 0.031 − osmotic factor of drug prescribed.

The number of Gm. of substance to be added for 100 cc. of solution is calculated as follows (*C*):

$$\text{Number of Gm. of substance to be added for 100 cc.} = \frac{\left(\begin{array}{c}\text{Osmotic factor of}\\ \text{substance to be}\\ \text{added}\end{array}\right)\left(\begin{array}{c}\text{Gram-molecular}\\ \text{weight of substance}\\ \text{to be added}\end{array}\right)}{\left(\begin{array}{c}\text{Number of particles from one}\\ \text{molecule of substance to be added}\end{array}\right)}$$

For nonelectrolytes and feebly ionized substances such as boric acid the number of particles from one molecule is taken as 1. For strong electrolytes, complete dissociation is assumed and the number of particles is taken to be the same as the number of ions into which the compound dissociates.

Since complete dissociation is assumed for sodium chloride in formula (*A*), this compensates for part of the error incurred in assuming complete dissociation of strong electrolytes in formula (*C*). The error in assuming complete dissociation does not exceed 5%, provided the solution does not have a concentration greater than 0.1 molar (2). The method is thus satisfactory for practical work. One advantage of the method is that it is established on a weight/volume basis, which is more convenient for pharmacists than the weight/weight method of Raoult's law.

Example 3: Prepare 100 cc. of a 1% solution of cocaine hydrochloride using sufficient sodium chloride to make the solution isotonic with the blood.

Substituting in formula (*A*), we have: osmotic factor for cocaine hydrochloride = $2 \times 1/339.7 = 0.006$.

Using this result in formula (*B*), we get: osmotic factor of substance to be added = 0.031 − 0.006 = 0.025.

Substituting in formula (*C*): Number of Gm. of sodium chloride to be added in 100 cc. = $0.025 \times 58.5/2 = 0.73$. Therefore 0.73 Gm. of sodium chloride would be used in making 100 cc. of the solution.

In 1916, Zotier (24) described a method for calculating the concentration of isotonic solutions based on comparison with the number of dissolved particles (molecules or ions) contained in a solution of sodium chloride containing 9.5 Gm. of NaCl per L., the latter solution being considered isotonic with the blood. The isotonic coefficients of substances were taken from the early work of De Vries. By

formulating the expressions as a mathematical series for a variable number of substances, Zotier derived formulas which could be used to solve special types of problems involved in preparing isotonic solutions containing several different solutes, such as: (*a*) the molecular concentrations of the solutes shall be equal, (*b*) equal weights of the solutes shall be used, (*c*) the solutes shall be present in definite ratios as 3, 5, 7, etc. Zotier's work has the error that the calculations are made to the fourth decimal place (fourth significant figure), while the isotonic coefficients employed are not accurate to the first decimal place (second significant figure). The calculations thus give an illusion of extreme accuracy which is not justified, the real errors being more than a hundred times as great as would be inferred from the number of decimal places or significant figures employed in expressing the results.

Nicola's Method.—To calculate the weight of any substance required for making one liter of solution isotonic with the blood serum, which was taken to be isotonic with 0.95% NaCl solution, Nicola (8) used the following formula: $p = 0.302\ m/i$, where p = Gm. per liter, i = isotonic coefficient and m = molecular weight. The following approximate values are used for i: 1 for nonelectrolytes, 1.5 for electrolytes which give two ions, 2.0 for electrolytes which give three ions and 2.5 for electrolytes which give four ions. These values of i used by Nicola were based on the results obtained by De Vries in his early work on plasmolysis. Nicola's method is the same as that previously described by Zotier, except that Zotier used a value of 1.5 instead of 1.86 for the isotonic coefficient of NaCl.

Example 4: Calculate the concentration of an isotonic solution of sucrose. For sucrose, $m = 342$ and $i = 1$; therefore $p = 0.302 \times 342/1 = 103.28$ Gm. per liter.

Example 5: To render a 1% solution of cocaine hydrochloride ($m = 339.5$, $i = 1.5$) isotonic by addition of NaCl ($m = 58.5$, $i = 1.86$), Nicola calculates as follows: $10 \times 1.5/339.5 = 0.0441$ and $(0.302 - 0.0441)58.5/1.86 = 8.1$ Gm. per liter.

The result in example 5 is 8.1 Gm. NaCl per liter as compared with an experimentally determined (9) value of 7.4 Gm.

There is also an inconsistency in using a value of i for NaCl of 1.86 and a figure of 1.5 for other electrolytes which yield two ions.

For solutions isotonic with the tear secretion, Nicola's formula is: $p = 0.43\ m/i$.

Mellen and Seltzer (10) have used Nicola's method in compiling tables of sodium chloride tonicic equivalents of various drugs used in ophthalmic prescriptions. The use of these tables shortens the calculations. For convenience in dispensing, Mellen and Seltzer suggest that the pharmacist may keep on hand isotonic solutions of various drugs. Then in dispensing it will only be necessary to measure the quantity of isotonic solution of each drug prescribed representing the number of grains required and to add isotonic solution of sodium chloride to bring to the volume specified. However, there are some disadvantages in this procedure. Thus in some cases, *e. g.*, borax, an isotonic solution of the substance alone cannot be prepared, because the proportion required exceeds the solubility. Stock solutions of some of the drugs are impracticable because of deterioration.

Graphical Method.—Nixon (5) has described a graphical method intended to avoid the repetition of calculations for solutions of different percentage strengths. In the construction of the graph, two factors must be determined: (*a*) the *w/v* percentage of NaCl or other adjusting agent necessary to produce a solution isotonic with the blood serum or lachrymal secretion, and (*b*) the *w/v* percentage of the drug solution which is isotonic with the blood or tear secretion. Nixon calculated these values on the basis of the osmotic factor method, which was used in the form outlined by Brown (6). Value *a* is placed on the graph on one axis and value *b* is placed on the other axis. The fixed points are joined by a straight line. It is then possible to obtain, by direct reading from the graph, the percentage of adjusting agent which must be added to a given percentage solution of the drug in order to obtain an isotonic solution.

Fahmy (7) constructed similar graphs in which a number of drugs were included in each chart, the concentration of drug solution being plotted against the freezing-point lowering.

Freezing-Point Method.—The quantity of a substance required to make a given solution isotonic with the blood may be calculated by the method of Lumière and Chevrotier, as follows (11): $X = (0.56 - A)/B$, where X = number of Gm. of substance to be added for 100 cc. of the solution, A = freezing point of the unadjusted solution in degrees below 0° C., and B = freezing point of a 1% solution (*w/v*) of the substance to be added. This formula is included in the latest editions of the Swiss Pharmacopœia and British Pharmaceutical Codex.

The value of B has been determined experimentally for an umber of drugs. The value of A may be determined experimentally or it may be calculated by multiplying the strength of the solution expressed as percentage *w/v* by the value of B for the substance. In the latter calculation an error may arise, for example, if the freezing-point lowering of a 10% solution is not exactly ten times that of a 1% solution.

An analogous formula used in calculating the concentration of solutions isotonic with the tear secretion is: $X = (0.80 - A)/B$.

A table of the freezing-point lowering of various drugs and examples of calculations are given in the experimental part of the present paper.

Böhme's Method.—Böhme (2) has developed a method in which the freezing point of the drug solution is calculated, assuming complete dissociation of strong electrolytes, and the quantity of adjusting substance required to produce the remainder of the

freezing-point lowering is obtained by interpolation from a table of experimentally determined freezing points of solutions of various concentrations. The table includes a number of adjusting substances from which the pharmacist can make an appropriate selection. By using experimentally determined figures for the adjusting substance, the error caused by assuming complete dissociation is limited to the drug itself. For solutions not exceeding 0.1 molar concentration, the error has a maximum of 5%.

Modified Freezing-Point Method.—Another method (25) involves the use of a modification of Raoult's law, the value of i being taken from a table in which electrolytes are classified according to the number of ions and the nature of the negative ion.

EXPERIMENTAL

Experimental studies of isotonic solutions of a number of substances used in pharmacy have been

TABLE I.—FREEZING-POINT LOWERING OF 1% *W/V* SOLUTIONS IN ° C.[a]

Alum (potassium)	$AlK(SO_4)_2.12H_2O$	0.11
Ammonium chloride	NH_4Cl	0.655
Antipyrine	$C_{11}H_{12}ON_2$	0.098
Atropine sulfate	$(C_{17}H_{23}O_3N)_2.H_2SO_4.H_2O$	0.08
Boric acid	H_3BO_3	0.315
Caffeine	$C_8H_{10}O_2N_4.H_2O$	0.075
Calcium chloride[b]	$CaCl_2$	0.42
Calcium gluconate	$C_{12}H_{22}O_{14}Ca.H_2O$	0.082
Calcium lactate	$Ca(C_3H_5O_3)_2.5H_2O$	0.12
Cocaine hydrochloride	$C_{17}H_{21}O_4N.HCl$	0.11
Cupric sulfate	$CuSO_4.5H_2O$	0.09
Dextrose	$C_6H_{12}O_6.H_2O$	0.095
Emetine hydrochloride	$C_{29}H_{40}O_4N_2.2HCl$	0.082
Ephedrine hydrochloride	$C_{10}H_{15}ON.HCl$	0.16
Ephedrine sulfate	$(C_{10}H_{15}ON)_2.H_2SO_4$	0.11
Ethylmorphine hydrochloride	$C_{19}H_{23}O_3N.HCl.2H_2O$	0.093
Glycerin	$C_3H_5(OH)_3$	0.205
Homatropine hydrobromide	$C_{16}H_{21}O_3N.HBr$	0.11
Lactose	$C_{12}H_{22}O_{11}.H_2O$	0.06
Magnesium chloride[b]	$MgCl_2.6H_2O$	0.245
Magnesium sulfate	$MgSO_4.7H_2O$	0.115
Mercuric chloride	$HgCl_2$	0.07
Mercuric cyanide[b]	$Hg(CN)_2$	0.08
Mercuric succinimide	$Hg(C_4H_4O_2N)_2$	0.08
Methenamine	$C_6H_{12}N_4$	0.14
Mild protein silver (Argyrol)		0.11
Morphine sulfate	$(C_{17}H_{19}O_3N)_2.H_2SO_4.5H_2O$	0.07
Phenol	C_6H_5OH	0.20
Physostigmine salicylate	$C_{15}H_{21}O_2N_3.HC_7H_5O_3$	0.11
Pilocarpine hydrochloride	$C_{11}H_{16}O_2N_2.HCl$	0.125
Potassium chloride[c]	KCl	0.485
Potassium iodide	KI	0.22
Potassium nitrate	KNO_3	0.35
Procaine hydrochloride	$C_{13}H_{20}O_2N_2.HCl$	0.14
Quinine hydrochloride[c]	$C_{20}H_{24}O_2N_2.HCl.2H_2O$	0.09
Quinine and urea hydrochloride	$C_{20}H_{24}O_2N_2.HCl.CO(NH_2)_2.HCl.5H_2O$	0.15
Silver nitrate	$AgNO_3$	0.225
Sodium benzoate	$NaC_7H_5O_2$	0.26
Sodium bicarbonate	$NaHCO_3$	0.40
Sodium biphosphate	$NaH_2PO_4.H_2O$	0.265
Sodium borate	$Na_2B_4O_7.10H_2O$	0.25
Sodium bromide	$NaBr$	0.36
Sodium cacodylate	$Na(CH_3)_2AsO_2.3H_2O$	0.175
Sodium chloride	$NaCl$	0.58
Sodium citrate	$Na_3C_6H_5O_7.2H_2O$	0.185
Sodium iodide	NaI	0.22
Sodium nitrate[d]	$NaNO_3$	0.36
Sodium salicylate	$NaC_7H_5O_3$	0.23
Sodium sulfate[d]	$Na_2SO_4.10H_2O$	0.165
Sodium thiosulfate	$Na_2S_2O_3.5H_2O$	0.178
Soluble iodophthalein	$C_{20}H_8O_4I_4Na_2.3H_2O$	0.07
Soluble phenobarbital	$C_{12}H_{11}O_3N_2Na$	0.155
Strong protein silver (Protargol)		0.02
Strong protein silver (Mallinckrodt's)		0.055
Sucrose	$C_{12}H_{22}O_{11}$	0.055
Tannic acid		0.02
Urea[d]	$CO(NH_2)_2$	0.31
Zinc sulfate	$ZnSO_4.7H_2O$	0.09

[a] Unless otherwise indicated, the substances used were of U. S. P. XI quality.
[b] Analytical reagent grade.
[c] N. F. VI quality.
[d] C. P. quality.

carried out by Poehl (12), Bennett and Gamble (13), Lumière and Chevrotier (11), Hattie (14), Van Itallie (9), Böhme (2), Nixon and Culbert (3), Whittier (15), Pinschmidt and Krantz (16), Picon (17), Dalimier (18) and Hirschlaff (19). Freezing-point data for solutions of some chemicals may also be found in the International Critical Tables, Smithsonian Tables and Landolt-Börnstein Tabellen. Such experimental data are frequently given in tables along with other less dependable data obtained by calculation. For this reason and because of the discrepancies in freezing points given in the literature by various authors, it was considered desirable to check the freezing-point determinations for a number of drugs, as well as to determine the freezing points of solutions of some drugs not previously recorded in the literature.

Apparatus.—The Beckmann freezing-point apparatus and thermometer were employed.

Materials.—In order that the results would be directly applicable to practical compounding, the drugs and chemicals used were of U. S. P. XI quality as a general rule, with a few exceptions in which the substances were of C. P. grade. In every case the material was taken from a previously unopened container in order to minimize the possibility of contamination or absorption of excess moisture.

Preparation of Solutions.—Solutions were prepared on a *w/v* basis, the substance usually being dissolved at room temperature and the solution brought to volume in a 100-cc. volumetric flask. If necessary, heat was used to hasten solution, and the liquid brought to 20° C. before adjusting to volume.

Procedure.—An approximate determination of the freezing point of each solution was first made, the solution being cooled rapidly by direct contact with the freezing mixture of ice, water and salt. A more accurate determination was then made by slowly cooling the solution, which was protected from direct contact with the freezing mixture by means of an air jacket. No correction was made for supercooling, since it was found convenient to avoid excessive supercooling by the addition of a small crystal of ice when the temperature had fallen a few tenths of a degree below the value obtained in the preliminary approximate test.

The experimental data obtained are given in Table I, the figure given for each substance being the average of at least three concordant determinations.

Examples of Calculations.—The use of the freezing-point method in preparing isotonic solutions is shown in the illustrations which follow.

Example 6: Prepare 100 cc. of a 3% solution of cocaine hydrochloride, using sufficient sodium chloride to make the solution isotonic with the blood.

Table I indicates that the freezing-point lowering of a 1% solution of cocaine hydrochloride is 0.11° C. Accordingly, the value of A (freezing-point lowering of a 3% solution of cocaine hydrochloride) is calculated as follows: $3 \times 0.11 = 0.33$. The value of B for sodium chloride as given in Table I is 0.58. The values of A and B are substituted in the formula. $X = (0.56 - A)/B = (0.56 - 0.33)/0.58 = 0.23/0.58 = 0.40$. Therefore 0.40 Gm. of sodium chloride would be used in making 100 cc. of the solution.

Example 7: Prepare 100 cc. of a 1% solution of silver nitrate, using sufficient sodium nitrate to make the solution isotonic with the tear secretion.

Table I indicates that the freezing-point lowering of a 1% solution of silver nitrate is 0.225 and that the value of B for sodium nitrate is 0.36. The values of A and B are substituted in the formula. $Y = (0.80 - A)/B = (0.80 - 0.225)/0.36 = 0.575/0.36 = 1.6$. The quantity of sodium nitrate to be used for 100 cc. of solution is 1.6 Gm.

Concentration of Isotonic Solutions.—On the basis of a survey of the literature, supplemented by experimental determinations in numerous instances, data were compiled regarding the percentage strength of isotonic solutions of various drugs. The results are presented in Table II. In using this table, it must be understood that these results are presented as numerical data, with no recommendations regarding possible medical use of the solutions. In some instances the solutions would not be suitable for medical use as such, *e. g.*, a 4.31% solution of silver nitrate would not be used in the eyes, but such solutions might be used in compounding.

TABLE II.—CONCENTRATION OF ISOTONIC SOLUTIONS[a]

Substance	Percentage Strength (*w/v*) of a Solution Isotonic With Blood (Δ = 0.56° C.)	Percentage Strength (*w/v*) of a Solution Isotonic With Tear Secretion (Δ = 0.80° C.)
Boric acid	1.8	2.4
Calcium gluconate	10.0	....
Calcium lactate	4.32[b]	....
Dextrose, anhydrous	5.1[b]	....
Lactose	9.5	....
Magnesium sulfate, crystalline	6.35[b]	....
Phenol	2.94[b]	....
Potassium chloride	1.12[b]	1.77[c]
Potassium nitrate	....	2.60[c]
Potassium sulfate	2.11[b]	....
Procaine hydrochloride	5.0	....
Silver nitrate	....	4.31[c]
Sodium benzoate	2.20	....
Sodium bicarbonate	1.4	2.0
Sodium borate	2.89[b]	4.10[c]
Sodium bromide	1.77[b]	....
Sodium cacodylate	3.3	....
Sodium chloride	0.95	1.40
Sodium citrate	3.0	....
Sodium iodide	2.5	3.58[c]
Sodium nitrate	1.4	2.05
Sodium phosphate, acid	2.64[b]	....
Sodium phosphate	4.53[b]	....
Sodium salicylate	2.4	....
Sodium sulfate ($Na_2SO_4.10H_2O$)	....	6.30[c]
Sucrose	10.0	....
Urea	1.8	....

[a] Unless otherwise indicated, the figures listed are experimental results of the present investigation.
[b] Figure taken from reference 13.
[c] Figure taken from reference 2.

DISCUSSION OF RESULTS

Freezing-Point Method.—The accuracy of the results obtained with the Beckmann apparatus is dependent on several factors. The rate of cooling is important, consequently care should be taken to obtain the same slow rate of cooling in all experiments. It is also necessary to avoid alterations due to absorption or evaporation of moisture, and to take care that the stirring takes place in the interior of the solution. Supercooling must be minimized or a correction made. Errors arise in the freezing-point method in case of some solutions in which the pure solvent does not separate on freezing. Difficulties are encountered in some instances in which the solubility of substances at 0° C. is much less than at room temperature.

An error may be introduced if the degree of dissociation or association at 0° C. is not the same as a body temperature. Other disadvantages of the freezing-point method are that data are available for only a limited number of substances and in some cases different values are given for the same substance in different tables (23).

Since Raoult's law is valid only for dilute solutions, an error arises in application of the law to solutions of higher concentration (22).

Some advantages of the freezing-point method are that it is suitable for all kinds of substances and is the only method which can be applied for substances of unknown molecular weight. An important advantage over methods of calculation is that the freezing-point method takes count of all changes in solution such as association, dissociation, common ion effect and other phenomena.

As previously stated, there is a chance for some error to be introduced in assuming that the freezing points of more concentrated solutions are proportional to the freezing point of a 1% solution.

TABLE III.—FREEZING-POINT LOWERING OF SOLUTIONS OF PROCAINE HYDROCHLORIDE

Concentration of Solution (*w/v*), %	Freezing-Point Lowering	
	Calculated	Determined
1	....	0.14
2	0.28	0.255
5	0.70	0.54

As indicated in Table III, the results for procaine hydrochloride show that the freezing point of a 2% solution is not exactly twice that of a 1% solution and that the freezing point of a 5% solution is not five times that of a 1% solution. For drugs used in percentage strengths considerably removed from 1%, the accuracy would be improved by making experimental determinations of the freezing points of solutions of approximately the same concentration as that of the solutions used.

Form of Substance Used.—In all methods of determining the concentration of isotonic solutions, it is important to note whether the substance to be used is in the anhydrous form or in the form of a hydrate. Thus some authors have published data for zinc sulfate based on the anhydrous form, $ZnSO_4$, while others have used the same words "zinc sulfate" as applying to the official salt which is a hydrate, $ZnSO_4.7H_2O$. Needless to say, considerable errors may arise when the water of crystallization is not taken into consideration. To avoid these errors, the data in Table I in the present paper include the chemical formulas showing the exact number of molecules of water of crystallization in the samples used for the determinations.

The results in Table I indicate that the freezing points of 1% solutions of strong protein silver are different for different brands. Hence the freezing-point data obtained with one brand of protein silver cannot be applied to another brand.

Comparison of Methods.—It is of interest to compare the results obtained by different methods for the same solution.

Example 8: Prepare 100 cc. of a 1% solution of cocaine hydrochloride, using sufficient NaCl to make the solution isotonic with the blood. The results by various methods are as follows:

Method	Gm. NaCl
Experimental (freezing-point method)	0.74
Brown's formula	0.75
Osmotic factor method	0.73
Graphical method	0.75
Nicola's formula	0.81
Lumière and Chevrotier's formula	0.78
Böhme's method	0.77

The figures indicate that for a dilute solution of an alkaloidal salt, the results by a number of methods are fairly concordant for practical purposes. For salts giving two divalent ions and for more concentrated solutions of various compounds, the differences between the results obtained by various methods are greater.

In methods involving the use of the isotonic coefficient, a certain degree of error is always encountered in arriving at the value of *i*. The results on the value of *i* for the same substance are not exactly the same as determined by different methods, *e. g.*, freezing-point method, conductivity and direct determination of osmotic pressure (22). Application of mathematical formulas may lead to error where there are several solutes which may have mutual effects such as common ion effect, complex formation and changes in association or hydration (23).

In using the graphical method, a slight error arises from the fact that the graphs are drawn as straight lines, but actually the freezing-point depression may not be a linear function of the concentration (23). Furthermore the graphical method has the errors which are present in the data used in constructing the graphs.

Accuracy Required in Practical Work.—In considering isotonic solutions, it is common to take the freezing point of blood as −0.56° C. However, the freezing point of human blood in health may

vary between −0.49° C. and −0.59° C. (20). Because of this variation in natural blood, it is evident that extreme accuracy is not required in isotonic solutions for practical purposes.

For the tear secretion, most authors accept the freezing point as being −0.80° C. A few references give the freezing point of tears as −0.85° and −0.86° C., but practically all state that 1.4% solution of sodium chloride is isotonic with the tear secretion and such a solution of NaCl has a freezing point of −80° C.

General Rules.—A few general rules may be stated as follows:

Rule 1: Where the concentration of drug is 0.005 molar or less, the osmotic pressure of the drug may be neglected. In such cases a solution isotonic with the blood may be prepared by dissolving the drug in an isotonic vehicle such as physiological solution of sodium chloride, or a solution isotonic with the tear secretion may be made by dissolving the drug in a 1.4% solution of NaCl.

Rule 2: Where the concentration of drug is 0.1 molar or less, the calculation may be made by the osmotic factor method or freezing-point method or a combination of the two (Böhme's method). For strong electrolytes, full dissociation may be assumed in the osmotic factor method, the number of ions being deduced from the chemical formula of the compound.

Rule 3: Where the concentration of drug is greater than 0.1 molar, the freezing-point method is preferable.

Rule 4: Where the exact molecular weight of the drug is unknown, the freezing-point method may be used.

SUMMARY

The theoretical foundation for the preparation of isotonic solutions is outlined and methods for calculating the concentration of isotonic solutions are described. The advantages and limitations of the various methods are discussed.

New experimental data are given on the freezing points of solutions of a number of drugs. The application of the data in practical pharmaceutical work is illustrated by examples.

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