Review Article

iMedPub Journals
http://www.imedpub.com/

Journal of Pharmacy Practice and Education

2018
Vol.1 No.1:6

Tonicity Calculations: An Aid to Understanding

Valter Travagli*

Associate Professor of Pharmaceutical Technology and Chief of the Post-Graduate School of Hospital Pharmacy, Dipartimento di Biotecnologie, Chimica e Farmacia, Via Aldo Moro, 2 53100 Siena, Italy

***Corresponding author:** Valter Travagli, Associate Professor of Pharmaceutical Technology and Chief of the Post-Graduate School of Hospital Pharmacy, Dipartimento di Biotecnologie, Chimica e Farmacia, Via Aldo Moro, 253100 Siena, Italy, Phone: +39 0577 234317; Fax: +39 0577 234258; E-mail: valter.travagli@unisi.it

Received date: December 12, 2017; **Accepted date:** January 18, 2018; **Published date:** January 22, 2018

Citation: Travagli V (2018) Tonicity Calculations: An Aid to Understanding. J Pharm Prac Edu Vol.1, No.1:6.

Abstract

Isotonicity is an important requisite mainly for parenteral aqueous-based pharmaceutical preparations. In addition, ophthalmic, nasal, auricular dosage forms together with irrigations meet such a requirement. The main purpose of this report is to present an advanced informative support to a challenging subject in relation to pharmaceutical formulation. The understanding of the theoretical fundamentals professionally distinguishes the work of the pharmaceutical formulator, which in this way has la possibility to put into practice such information with more knowledge. Such a feature is of certain concern from an informative perspective when specific software or online facilities are used to solve tonicity problems.

Firstly, this paper will give a precise definition of the concepts used. Then, it will introduce the osmometry from a pathophysiological outlook, in order to set such an issue in the context of its practical implications. The necessity to achieve isotonicity will then be dealt with by illustrating the rational processes, including arithmetic calculations, involved in the production of accurate formulations. A critical presentation of the various methods of adjusting the tonicity of a solution (ΔT_f, osmotic factor, Equivalent method, V-value) with the use of some simple applied examples will be reported.

Keywords: Isotonicity; Pharmaceutical calculation; Colligative properties

Introduction

The tonicity of a solution may be defined as the characteristic represented by the effects that the solution has on the morphology of the cells that it comes into contact with. In fact, the subject of tonicity deals with modifications of the "tone" of the cells. i.e. of the normal morphological state of a cell following contact with a solution containing a given concentration of dissolved particles, regardless of the nature (e.g. species, charge state, dimension) of the particles. In short, all pharmaceutical preparations in which the absence of "insults" referable to phenomena of osmosis is important for physiological purposes must meet the requisite of not provoking morphological variations in the cells they meet. As we know, the term "osmosis" theoretically identifies the diffusive passage of a solvent (usually water) through a membrane that is only permeable to the solvent (ideal semipermeable membrane) situated between two solutions with different concentrations of particles of solutes. From a clinical point of view, the phenomenon belongs entirely to the concept of homeostasis, and has been dealt with in various works [1,2]. However, there do not seem to be any works in the literature, that deal with the problem in an exhaustive and/or, at the same time, practical manner from a formulation perspective [3-5]. The pharmaceutical preparations affected by the phenomenon of osmotic tolerance are aqueous preparations [6-8]:

- For ophthalmic use, where a lack of isotonicity leads to irritation or, as in the case of contact lenses, sticking to the eye, burning, dryness, photophobia;
- For parenteral use, where the effects largely depend on the degree of deviation from isotonicity, the site of administration, the quantity administered and the method of administration;
- For auricular use;
- For nasal use;
- For irrigation, especially in the treatment of very deep and extensive wounds.

It is important to bear in mind that, although it is important to adjust the tonicity as precisely as possible (in the blood, for example, extreme hypertonicity leads to shrinkage and plasmolysis, while marked hypotonicity presents the phenomenon of swelling, with possible loss of intracellular material and haemolysis in extreme cases), this requisite still leaves the formulator a margin for manoeuvre. The fact that the cells tolerate slightly hypertonic preparations better than slightly hypotonic preparations may also be taken into consideration **(Figure 1).**

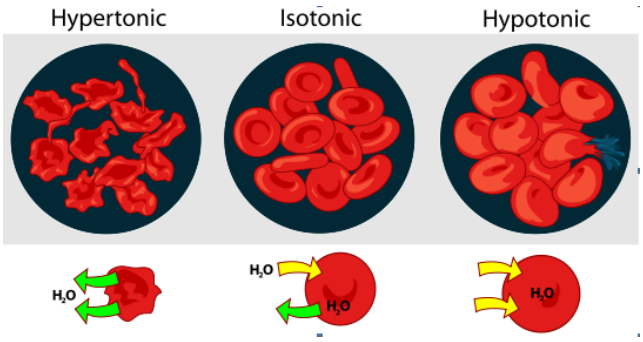

Figure 1: Schematic representation of erythrocyte behaviour in hypertonic (left); isotonic (middle); hypotonic (right) solutions

Furthermore, the cell membrane does not in reality behave as an ideal semipermeable membrane towards solvents or towards some of the particles dissolved in them. For this reason, a differentiation exists in the terminology between iso-osmoticity, which identifies a solution characterised by the same number of dispersed particles, and isotonicity, which implies the maintenance of morphological characteristics of cells with respect to a reference solution. A solution can thus be iso-osmotic without necessarily having to be isotonic [9,10]. In this sense it is useful to bear in mind that as well as water, other solvents and co-solvents, such as ethanol, glycerine and propylen glycol, are used as drug delivery vehicles. These compounds also contribute to altering some of the properties of the resulting solutions, although it is not possible to know in advance what their contribution will be in terms of tonicity [11,12]. For this reason, all the aspects that are dealt with below are to be considered applicable to solutions with pharmaceutical grade water as their only solvent.

As previously indicated, the physiological evaluation of the condition of isotonicity (also identified in terms of osmotic tolerance) by the observation of morphological changes in the erythrocytes differs from quantitative evaluation by means of analytical methods based on osmotic pressure, i.e. the pressure which would need to be exerted to halt the diffusion of water through a semipermeable membrane [13].

This aspect is very important at a theoretical level, in order to understand the typical properties of the solutions in terms of analogies with the concepts of ideal gas behaviour, as previously described by J. H. van't Hoff at the end of the nineteenth century and thus develop the reasoning processes necessary to identify adequate formulation criteria [14]. In summary, the passage in a solution of one or more solutes in a given solvent (in this case, water) leads to:

- i) At equilibrium, a homogeneous solution of the solute molecules in the total available liquid;
- ii) In a solution containing a number n of solutes, each solute behaves as if it were the only solute in that solution (cf Dalton's law). Furthermore, although the analogy with ideal gases is only formal, osmotic pressure depends on the available volume, the number of moles of solute, universal gas constant, and absolute temperature.

The essential difference with ideal gases lies in the fact that for them the system pressure is directly generated by the gas molecules, while in the case of solutions it is generated by the variation of free energy in the system, i.e. in relation to the number of particles formed during the process of solubilization. Before we deal with the technological aspects of the formulation of pharmaceutical preparations that meet the requisite of tonicity, we should bear in mind that for some preparations osmotic characterization represents a topic of physiopathological interest that regards total parenteral nutrition (TPN), enteral hyperalimentation, infant formula preparation, diagnosis of pathological conditions at the level of urinary tract and other secretion sites. Furthermore, some medicinal products are based upon osmotic action for therapeutic effects. In fact, there are numerous oral and parenteral commercial preparations based upon hypertonicity [15].

These include intravenous mannitol 20% to promote osmotic diuresis; urea 30% to reduce intracranial and intraocular pressure; the same urea but at a concentration of 40-50% for the termination of pregnancy; sodium chloride 1.8-5% in the treatment of severe acute dilutional hyponatraemia (and sodium chloride 20% for the termination of pregnancy, like urea). Furthermore, glucose 25-50% is used in peritoneal dialysis, although nowadays other hypertonic dialysates such as lactate and bicarbonate are preferred. Finally, glycerol 50%, isosorbide up to 70-80% and up to 10 g magnesium sulphate or magnesium hydroxide are also used per os.

Another aspect regarding the potential of osmotic pressure that involves both the pharmaceutical industry and academic structures is its technological application in the field of therapeutic systems in modified-release drugs. In fact, osmotic pressure can be employed as the driving force in a device in order to obtain osmotic pumps as a useful device in drug delivery [16-19].

It is also necessary to deal with conditions of hypertonicity in the case of parenteral preparations for intramuscular administration that contain a greater quantity of active principle than necessary to achieve isotonicity (e.g. some β-lactam antibiotics).

However, professionals whose work is dedicated to the formulation of pharmaceutical preparations characterised by isotonicity will be specifically interested in methods for rendering isotonic solutions that would otherwise be hypotonic because of the quantity of particles dissolved in them. The preparation by the hospital pharmacist of specific pharmaceuticals such as radiopharmaceuticals is an example that illustrates the current importance of this topic. In such cases, it is important to possess a knowledge of the physical properties of a solution, which depend on the number of particles present and not on their chemical properties. These properties are classified as *colligative* **properties** (i.e. a group of properties *collected together*), a third category that is a subset of a system's intensive properties and that can only be applied

to solutions. By definition, one of the properties of a solution is a *colligative property* if it depends only on the ratio of the number of particles of solute and solvent in the solution, not on the identity of the solute. They take their name from the fact that they represent different manifestations of a single thermodynamic phenomenon: the decrease of vapour pressure of a solvent due to the presence of a non-volatile solute. It can also be affirmed that if the measurement of one of the colligative properties (i.e. osmotic pressure, boiling point elevation, freezing point depression) in solutions with different solutes provides the same values, this equality between the two solutions will also be encountered in the measurement of other colligative properties. Leaving aside a detailed explanation of the thermodynamic and chemical aspects such as the derivation of the dissociation factor *i* (or the van't Hoff factor in its explicit form)[a], the derivation of the freezing-point depression constant (K_f)[b], and the definition of molality (m)[c], it is useful at this stage to start with the following consideration: iso-osmotic solutions (and, generally, isotonic solutions) must also have the same freezing and boiling temperatures. Furthermore, solutions containing the dissolved solute have a lower freezing temperature (T_f) and a higher boiling temperature (T_b) than pure solvent ($T_{0,f}$ and $T_{0,b}$, respectively). The difference in these values is always positive and is generally known as freezing-point depression ($\Delta T_f=T_{0,f}-T_f$) and boiling-point elevation ($\Delta T_b=T_b - T_{0,b}$), respectively. The answers to the two following questions: i) "Two aqueous solutions have different freezing-point depression values. If the first freezes at a higher temperature than the second, how can we define it in relation to the latter?" and ii) "Two aqueous solutions have different boiling-point elevation values. If the first boils at a higher temperature than the second, how can we define it in relation to the latter?" are therefore "hypotonic, i.e. with a lower concentration of dissolved particles" and "hypertonic, i.e. with a higher concentration of dissolved particles", respectively. Furthermore, amongst the following solutions at the given molality: Zinc chloride 0.20; Sodium chloride 0.25; glucose 0.30, the greatest osmotic pressure will be provided by the first compound because it presents the highest number of dissolved particles in solution.

As regards the technological application of these remarks, the freezing point of lacrimal fluid or that of normal, healthy human blood is -0.52°C. Thus, an isotonic solution has a freezing-point depression value of 0.52°C.

This paper deals with the various methods that can be used to adjust the tonicity of a solution. We stress the fact that all the aspects dealt with below refer to water as the solvent and that reference to the colligative property represented by ΔT_f is the starting point for the following reasoning processes.

Practical Examples

Case of a substance from which to obtain an iso-osmotic solution

The relationship between freezing-point depression, the concentration of the solution and the number of particles formed by solubilisation is shown in Equation. 1:

$$\Delta T_f = K_f \cdot i \cdot m \quad \text{(Eq. 1)}$$

or, more explicitly

$$\Delta T_f = K_f \cdot \frac{w_{solute}}{M_{w\ solute}} \cdot [1 + \alpha \cdot (\nu - 1)] \quad \text{(Eq. 2)}$$

From Equation 2, and using our knowledge of the freezing-point depression, we can find the quantity of a generic substance necessary to obtain an isotonic solution. In fact, if we suppose that 1 kg of water is used as the solvent and solve Equation 2 for the amount of solute we obtain:

$$w_{solute} = \frac{\Delta T_f \cdot M_{w\ solute}}{K_f \cdot [1 + \alpha \cdot (\nu - 1)]} \quad \text{(Eq. 3)}$$

Thus, in 1 kg of water as a solvent for NaCl (knowing that α=0.93), anhydrous dextrose and dextrose monohydrate (ν=1, so i=0), considering the molecular weight of the substances specified (M_w) and remembering that ΔT_f=0.52°C, K_f=1.86, the values of 8.5, 50 and 55 grams were obtained, respectively[d].

Case of an active principle in a solution with a therapeutic concentration generally insufficient to achieve isotonicity

Let us move on to the most common situation encountered, namely when the quantity of active principle necessary to carry out the therapeutic task involves the realization of a hypotonic solution. In this case, we need to add auxiliary substances as isotonising agents. There are various methods of adjusting tonicity that make it possible to solve this formulation problem. These methods deal with aspects that can be classified as follows to make them more easily identifiable: reference molality; cryoscopic method (or freezing-point depression method); sodium chloride equivalent method; isotonic solution V values.

Concept of reference osmolality

Considering the example of an isotonic solution for NaCl, we know that the reference molality in terms of osmotic effects (also known as osmolality) in the case of 1 kg of water is represented by

$$osm_{NaCl0.9\%} = osm_{ref} = \frac{9\ g}{58.44\ g/mol} \cdot [1 + 0.93 \cdot (2 - 1)] \cong 0.3$$

Furthermore, because the colligative properties depend on the number of the particles in solution and not on the nature of the particles, isotonicity is achieved when $osm_{drug}+osm_{tonicity\ agent}=0.3$ or, more explicitly:

$$\frac{w_{drug}}{M_{drug}} \cdot [1 + \alpha \cdot (\nu - 1)]_{drug} + \frac{w_{tonicity\ agent}}{M_{tonicity\ agent}} \cdot [1 + \alpha \cdot (\nu - 1)]_{tonicity\ agent} = 0.3 \quad (Eq.\ 4)$$

As we have said, Equation 4 is valid for 1 kg of solvent. However, it may be interesting to extend the reasoning process to a generic quantity of solvent. Moreover, considering that for pharmaceutical formulation purposes the concentrations of the solutions in question are diluted (<<1M), the approximation between the values of osmolality and osmolarity (volume of the solution expressed in L, V_L) is admissible. Thus, solving the equation in terms of the quantity of tonicity agent to be added, we obtain:

$$w_{tonicity\ agent} = \left\{0.3 \cdot V_L - \frac{w_{drug}}{M_{drug}} \cdot [1 + \alpha \cdot (\nu - 1)]_{drug}\right\} \cdot \frac{M_{tonicity\ agent}}{[1 + \alpha \cdot (\nu - 1)]_{tonicity\ agent}} \quad (Eq.\ 5)$$

As well as establishing the quantity of tonicity agent to be added, Equation 5 is interesting in that it allows us to affirm that: i) if we vary the final volume of the solution by a factor k, maintaining the percentage of a drug constant, the quantity of tonicity agent to be added to make the solution isotonic will vary by the same factor k; ii) if we wish to vary the percentage of the drug and keep the final volume of the solution constant, the quantity of tonicity agent will be characterised by a coefficient that is variable and inversely proportional to the quantity of the drug; iii) the result of the difference within the braces represents an estimation of the deviation from the isotonicity. In fact, the higher this value is, the more tonicity agent needs to be added. If we increase the amount of the drug, the value of this difference will obviously decrease. The limit to this effect is constituted by reaching a value of zero, meaning that isotonicity has been achieved by the drug alone present in the formulation. If we continue to increase the amount of the drug we will obtain negative values of the tonicity agent, meaning that the conditions of isotonicity have been exceeded and a hypertonic solution has been obtained due to the presence of the drug alone. Such a situation arises in some parenteral preparations for intramuscular use that contain a high dose of drug (β-lactam antibiotics are a typical example). Paradoxically, from a formulation point of view this situation is dealt with by adding an adequate quantity of local anaesthetic. Although this procedure worsens the situation in terms of hypertonicity, it decreases the suffering caused by the osmotic insult provoked in the injection site and makes the preparation easier for the patient to be tolerated.

Cryoscopic method or freezing-point depression method

The freezing-point depression method identifies the lowering of the freezing temperature corresponding to a certain % w/v of a substance. The concentration used (given in relative tables) is generally 1% and is indicated as D (or ΔT_f 1%). This value is dimensionally represented by a temperature [°C]. Considering that the lowering of the freezing temperature is directly proportional to the concentration of the particles present, we can see how the tabulated D value for sodium chloride is higher than 0.52[e]. The following example illustrates how to use this method. Supposing we need to prepare 100 mL of atropine sulphate 1% eye drops. The corresponding D value in the table is 0.074. This value represents the contribution that the presence of atropine sulphate compound makes to ΔT_f. Therefore, as we know that a physiological solution has a ΔT_f of 0.52°C, we need to add enough isotonising agent to achieve ΔT_f =(0.52-0.074)°C[f].

Sodium chloride equivalent method

This approach is very interesting from a formulation point of view, partly because of its theoretical implications. Briefly, sodium chloride equivalent-generally indicated as E-, can be defined as "the weight of sodium chloride that produces the same osmotic effect as 1 g of a given substance in 100 mL of solution". Below, we describe a method of obtaining these values. Based on the affirmation of "equivalent", from an osmotic point of view, we can say the following for sodium chloride:

$$\Delta T_{f\ NaCl} = K_f \cdot \frac{w_{NaCl}}{M_{w\ NaCl} \cdot V} \cdot [1 + \alpha \cdot (\nu - 1)]_{NaCl} \quad (Eq.\ 6)$$

and for a generic substance:

$$\Delta T_{f\ substance} = K_f \cdot \frac{w_{substance}}{M_{w\ substance} \cdot V} \cdot [1 + \alpha \cdot (\nu - 1)]_{substance} \quad (Eq.\ 7)$$

By dividing the two equations, considering that in isotonic conditions ΔT_f is a constant value, K_f is a constant, fixing V=100 mL, weight of the substance=1 g, introducing the symbol E as the quantity of sodium chloride which is "equivalent to 1 g of substance from an osmotic point of view", and simplifying the equation we obtain:

$$E = \frac{M_{w\ NaCl}}{M_{w\ substance}} \cdot \frac{[1 + \alpha \cdot (\nu - 1)]_{substance}}{[1 + \alpha \cdot (\nu - 1)]_{NaCl}} \quad (Eq.\ 8)$$

Thus defined, the value E is a univocal value. However, if we extend the concept to variable $\%_{w/v}$ concentrations of the different substances, these E values tabulated in the relative tables may show small variations due to the inter-ionic interactions that may occur in aqueous solutions [2]. This aspect is of particular interest with relation to the degree of dissociation,α slight variability at the different experimental conditions. We can demonstrate how to make silver nitrate 1% eye drops isotonic as an example. The E value of this substance is 0.33, that is, 1 g $AgNO_3$, osmotically corresponds to 0.33 g NaCl in a volume of 100 mL. Based upon these results, the amount of NaCl to be added (in g) is equal to the difference 0.9-0.33, i.e. 0.57 g. As previously indicated, if the difference is less than zero the solution is already hypertonic and cannot be adjusted without altering the concentration of the components.

However, at a formulation level, the chemical incompatibility between the two products should be highlighted because of the

consequent precipitate formation of silver chloride. In these cases a different tonicity agent may be used. Supposing we have sodium nitrate at our disposal, whose E value=0.68, we can say that

1 g $NaNO_3$: 0.68 g NaCl=X g $NaNO_3$: 0.57 g NaCl. Solving the equation we obtain 0.84 g $NaNO_3$.

Therefore, by extending the concept to any tonicity agent other than sodium chloride, the amount to be added can be derived from the following equation:

$$w_{tonicity\ agent} = \frac{w_{NaCl}}{E_{tonicity\ agent}} \text{(Eq. 9)}$$

V-value method

The problem is dealt with in a different manner with the following method. While so far we have considered the amount of tonicity agent to be added in relation to the final volume of the preparation, with this method we obtain the volume of water to be added to a certain quantity of drug in order to obtain an isotonic solution[g].

To gain a better understanding of this aspect we can try to derive the V-value as defined above from the sodium chloride equivalent taken into consideration previously. In fact, for a generic amount a of a certain substance we know that

1 g substance: E=a g substance:X g NaCl

from which we obtain X g NaCl=a g substance · E.

Moreover, we can also say that:

100 mL isotonic solution : 0.9 g NaCl=V-value : (a g substance · E), i.e.

V-value=**a** g substance · E · 111.1 (Eq. 10)

Once we have calculated the volume of water to be added using Equation 10, we can obtain any volume we require by adding the isotonic vehicle (generically indicated as diluting solution).

Recapitulation and Conclusion

The aim of this paper is to provide general rather than exhaustive information regarding the formulation issues that a pharmacist formulator may encounter. Furthermore, pharmacists can be encouraged to learn the instructional value of this argument in order to underlie the basic principles of the calculations. To recapitulate with a practical example, consider an ophthalmic preparation with a final volume of 10 mL which contains two drugs, A and B, the former at a concentration of 1% w/v, the latter in the amount of 75 mg. Regarding A, we know that for 0.3 g of drug we need 9.7 mL of water to achieve isotonicity, while for the same amount of B we require 5.3 mL. Based on this data, and hypothesising adjusting tonicity by adding sodium chloride instead of normal saline solution to obtain the required volume, we can formulate the following: firstly, we can set the proportions 0.3 g: 9.7 mL=0.1 g: X mL and 0.3 g: 5.3 mL=0.075 g: Y mL, for A and B, respectively. By solving this equation we obtain 4.5 mL (3.2 mL plus 1.3 mL) of water to be added in order to obtain isotonicity. Thus, approximately 50 mg of NaCl and 5.5 mL of water are required to render the remaining volume isotonic.

Footnotes

[a]In this context, it is sufficient to consider that i=[1+α (v-1)] where α represents the degree of dissociation of the solute (whose value will therefore be between 0 in the case of non dissociable substances and 1 for completely dissociable substances) and v represents the number of particles that are formed by dissociation.

[b]It is possible to show that $Kf = \frac{R \cdot T_f^2}{\Delta H_f \cdot n} \cong 1.86$ for water [K kg mol-1], where R is the molar gas constant, T is the absolute temperature, ΔHf is the enthalpy of fusion and n the mol of the solvent.

[c]The term molality refers to **the number of moles of solute dissolved in one kilogram of solvent.**

[d]It is interesting to note how, theoretically, a physiological solution contains less NaCl than those generally used (0.85% *vs.* 0.9%). The value 0.9% derives from early measurements of the freezing temperature of blood carried out with instruments available at the time (the first researchers actually found the freezing temperature of blood to be -0.56°C)[11]. The fact that, despite this, the slightly more concentrated solution is universally recognised as physiological may be justified by the osmotic tolerability of slightly hypertonic solutions and by the non-ideal behaviour of the cell membranes towards the various solutes mentioned above.

[e]This affirmation derives from the following proportion: 0.9% NaCl: 0.52°C=1% NaCl: D_{NaCl}, with D_{NaCl}=0.576.

[f]The quantity of isotonising substance to be added derives from the following proportion: 1% substance : $D_{substance}$=X $\%_{substance}$: 0.45°C. Hypothesising the use of NaCl, substituting the relative values and solving the proportion, 0.78 g will need to be added to obtain an isotonic solution.

[g]Theoretically the volume of isotonic solution to be obtained should be calculated by solubilizing a given quantity of substance in an exact volume of water. However, in practice, considering that in the case of pharmaceutical preparations the solutions are diluted (<<1M), the volume of water to be added closely corresponds to the amount of solution obtained.

[h]The reason why the quantity of 300 mg has been indicated for many substances is because the various tables refer to a final volume of 30 mL (1 fluid-ounce) in order to obtain a final percentage of the drug of 1% w/v.

References

1. Bourque CW (2008) Central mechanisms of osmosensation and systemic osmoregulation. Nat Rev Neurosci. 9(7):519-531.
2. Poon CY (2006) Tonicity, osmoticity, osmolality, and osmolarity. In: David B. Troy, Paul Beringer Eds. Remington: the science and

practice of pharmacy. 21th ed. Philadelphia: Lippincott Williams & Wilkins; 250-265.

3. Attwood D, Florence AT (2012) Physical Pharmacy, 2nd ed. London: Pharmaceutical Press.
4. Ghosh TK, Jasti BR (2005) Theory and Practice of Contemporary Pharmaceutics, Boca Raton, Florida, US, CRC Press.
5. Hareesh RM, Sambasivarao K, Rao BC (2016) Methods of adjusting tonicity and pH values of some drugs and substances. Int. J. Adv. Res. Biol. Sci. 3(10): 207-212.
6. Chapman DG (2014) Parenteral products. In: Rees JA, Smith I, Watson J. Pharmaceutical Practice, 5th Ed. Edinburgh, Elsevier.
7. Thompson JE, Davidow L (2003) A practical guide to contemporary pharmacy practice. 2nd ed. Baltimore: Lippincott Williams & Wilkins.
8. de Mello Júnior JF, de Godoy MO, de Andrade NA, Anselmo-Lima WT, Stamm AEC et al.(2013) Brazilian Academy of Rhinology position paper on topical intranasal therapy. Brazilian journal of otorhinolaryngology, 79(3): 391-400.
9. Doemling DP (1968) Isotonic vs Isosmotic Solutions: A Clarification of Terms. JAMA,203(3): 232-233.
10. Zhan X, Li H, Yu L, Wei G, Li C (2014) Determining osmotic pressure of drug solutions by air humidity in equilibrium method. Drug development and industrial pharmacy, 40(6): 758-761.
11. Strickley RG (2004) Solubilizing Excipients in Oral and Injectable Formulations. Pharm. Res. 21(2): 201–230.
12. Pinarbasi T, Sozbilir M, Canpolat N (2009) Prospective Chemistry Teachers' Misconceptions about Colligative Properties: Boiling Point Elevation and Freezing Point Depression. Chem. Educ. Res. Pract. 10(4): 273-280.
13. Seifarth CC, Miertschischk J, Hahn EG, Hensen J (2004) Measurement of serum and plasma osmolality in healthy young humans–influence of time and storage conditions. Clinical Chemistry and Laboratory Medicine (CCLM), 42(8): 927-932.
14. van't Hoff JH (1887) The role of osmotic pressure in the analogy between solutions and gases. Zeitschrift fur physikalische Chemie. 1: 481-508.
15. Witherspoon B, Ashby NE (2017) The Use of Mannitol and Hypertonic Saline Therapies in Patients with Elevated Intracranial Pressure: A Review of the Evidence. Nursing Clinics of North America 52(2): 249-260.
16. Theeuwes F (1975) Elementary osmotic pump. J Pharm Sci. 64: 1987-1991.
17. Patel H, Parikh VP (2017) An overview of Osmotic Drug Delivery System: an update review. International Journal of Bioassays 6(7): 5426-5436.
18. Sareen R, Jain N, Kumar D (2012) An insight to osmotic drug delivery. Curr. Drug Deliv. 9(3): 285-296.
19. Uchiyama T, Watanabe J, Ishihara K (2004) Implantable polymeric artificial pancreas. J Biomat Sci, Polymer Ed. 15: 1237-1262.