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Corresponding Author: Dr. Encarna Garcia-Montoya, Ph D

Corresponding Author's Institution: University of Barcelona

First Author: Diana Arbelaez Camargo, PhD Student

Order of Authors: Diana Arbelaez Camargo, PhD Student; Manel Roig; Encarna Garcia-Montoya, Ph D; Pilar pérez Lozano; Montserrat Minarro; Josep R Ticó; Josep M Suñé Negre

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Osmolality predictive models of different Polymers as tools in parenteral and ophthalmic formulation development.

Authors

Diana Arbelaez Camargo[a], Manel Roig Carreras[a], Encarna García Montoya[a,b], Pilar Pérez Lozano[a,b], Montserrat Miñarro Carmona[a,b], Josep Ramon Ticó Grau[a,], Josep Maria Suñé Negre[a,b], [b]

a. Pharmacy and Pharmaceutical Technology Department, Faculty of Pharmacy, University of Barcelona, Joan XXIII 27-32, CP 08028 Barcelona - Spain.

b. Pharmacotherapy, Pharmacogenetics and Pharmaceutical Technology Research Group, IDIBELL-UB, Duran i Reynals Hospital, 3a level, Gran Via de l'Hospitalet 199, CP 08908 Hospitalet de Llobregat, Barcelona - Spain.

Corresponding author

Diana Arbelaez Camargo

Pharmacy and Pharmaceutical Technology Department, Faculty of Pharmacy, University of Barcelona. Joan XXIII, 27-32, 08028 Barcelona – Spain.

Tel: (+34) 662 32 11 46

Fax: (+34) 93 4 02 45 46

Mail: diancrayolina@gmail.com

Abstract

During the development of parenteral dosage forms, different physicochemical studies are required to ensure stable, effective and safe formulations. The osmolality of this kind of dosage form should bear a close similarity to the body fluids to prevent local irritation, pain or even more significant side effects like endothelial damage. The osmotic studies performed in Polyethylene glycol 400 (PEG 400), Polyethylene glycol 4000 (PEG 4000), Poloxamer 407 (P407), Sodium Hyaluronate (SH), Chondroitin Sulphate Sodium (CS), Cremophor RH 40 (CRE40) and Polyvinyl alcohol (PVA) aqueous solutions, showed that the theoretical determination of the osmolality based on their molecular weight as the only determinant factor did not agree with the values obtained by the measurement of colligative properties such as the freezing point depression. The data obtained from this study and its analysis, provided predictive equations that can be used as tools in the primary development to estimate formulation´s osmolality at different concentrations; and its evolution over a period at the hypothetical worst-case scenario of storage temperature.

Key words

Osmolality, fitting model equations, parenteral pharmaceutical development, Polyethylene glycol 400, Polyethylene glycol, 4000 Poloxamer 407, Sodium Hyaluronate, Chondroitin Sulphate Sodium, Cremophor Polyvinyl alcohol.

1. Introduction

In parenteral preparations, attention should be given to osmolality to prevent local irritation, pain or endothelial damage. Polymers and surfactants are widely used in this kind of preparations (Mathew et al., 2017; Nguyen et al., 2015; Sivashanmugam et al., 2015; Thambi et al., 2017) to obtain desired pharmacokinetic characteristics such as prolonged release profiles; or to improve drugs solubility and bioavailability, considering the low aqueous solubility of most active pharmaceutical ingredients.

Osmolality (Osm) is a measure of the osmotic pressure exerted by a real solution across a semipermeable membrane. Like osmotic pressure, other colligative properties of a solution, such as vapour pressure lowering, boiling point elevation, and freezing point depression, are also directly related to the osmolality of the solution (The United States Pharmacopeial Convention, 2017). When the osmotic pressure of a solution is measured by passing it through a cell membrane and then compared to the blood plasma, we are referring to tonicity (Remington, 2013; Wang, 2015). The freezing point depression in comparison to pure water is a direct measure of the osmotic concentration, this method is commonly used in pharmaceutical development as a fast, easy and accepted method to determine the osmolality of ophthalmic and parenteral formulations.

Generally, parenteral, ophthalmic, otic and nasal preparations are preferably isotonic (290 mOsm/kg) or moderately hypertonic (500 mOsm/kg) (Nony et al., 2001). Hyperosmolality leads to a loss of water from the cells which causes cell shrinkage and an increase in cellular viscosity (Reinhart et al., 2015).

Among the wide range of polymers used in the preparation of different drugs, Polyethylene glycols (PEGs) with a general of formula of HOCH2(CH2OCH2)mCH2OH, are water soluble inert polymers essentially non-irritant to the skin. They are classified according to their structural formula and molecular weight. Within the typical polyethylene glycols with

pharmaceutical significance, the polyethylene glycol 400 (PEG 400) is used in parenteral preparations as a solubilising agent and polyethylene glycol 4000 (PEG 4000) is used in bowel preparation before colonoscopy, radiological procedures, or surgery (Martindale, 2016a; Paul J Sheskey, 2016a). Hydrogenated Castor Oil derivatives, best known as Cremophor, are a series of materials obtained by reacting varying amounts of ethylene oxide with either castor oil or hydrogenated castor oil. Cremophor RH 40 (Cre40) is a non-ionic solubilizer and emulsifying agent made by reacting one mole of hydrogenated castor oil with 40 moles of ethylene oxide, with a molecular weight of 2500 g/mol (BASF SE Care Chemicals Division Pharma Ingredients & Services, 2008; Information, 2010). It is used as a solvent in vehicles for oral and topical solutions (Paul J Sheskey, 2016b). Another well-known non-ionic triblock copolymer is the Poloxamer 407 (P407), with a molecular weight of 12,500 g/mol and formed by two hydrophilic blocks and one hydrophobic block covalently bonded, is primarily used as surfactant in pharmaceutical formulations as emulsifying or solubilizing agent (Paul J Sheskey, 2015a). It is of great interest, that its micellar rearrangement in solution produces changes in viscosity when changing its temperature (Viegas and Henry, 1998), making them ideal to confer thermoreversible properties to formulations and develop novel controlled release formulations (Arbelaez-Camargo et al., 2016). Hyaluronic acid (HA), a high-molecular-weight polymer of 10 000 000–100 000 000 Da, is formed by d-glucuronic acid and N-acetyl-d-glucosamine disaccharide units ("Sodium Hyaluronate: Pharmaceutical Excipients," n.d.). This used in ophthalmic and parenteral formulations because of its bioadhesive, therapeutic and biodegradation properties (Martindale, 2016b). Among the bio-polymers with bioadhesive properties, the Chrondroitin sulphate (CS) is a glycosaminoglycans and disaccharide polymer composed of equimolar amounts of d-glucuronic acid, d-acetylgalactosamine and sulphates in 10–30 disaccharide units (Mason, 2016). With a molecular weight of 463.36854 g/mol and an important therapeutic effect in joint cartilage and connective tissue, it is used in different formulations not just because of its therapeutic effect, but also, as a bioadhesive in ophthalmic and parenteral formulations. Polyvinyl alcohol is a non-ionic surfactant obtained by polymerisation of vinyl acetate followed by partial or almost complete hydrolysis of polyvinyl acetate. Different grades are available and they differ in their degree of polymerisation and their degree of hydrolysis, which determine the physical properties of the different grades. The mean relative molecular mass lies between 20000 and 150000 (Martindale, 2016c). It is commonly used to increase the viscosity of ophthalmic preparations thus prolonging contact of active ingredient with the eye, in sustained-release formulations for oral administration and transdermal patches (Handbook of Pharmaceutical Excipients and Edited by Paul J Sheskey, n.d.).

With the aim of predicting the osmolality of ophthalmic and parenteral formulations that contain the polymers cited above, we studied the osmolality of the different polymers at different concentrations and, over a period, we studied the hypothetical worst-case temperature scenario (40 ºC) of storage.

2. Material and methods

2.1. Materials

Polyglykol 400 (Polyethylene glycol 400 - PubChem CID: 174) manufactured by Clariant (Sulzbach) Germany, batch DEG4005530. Polyethylene glycol 4000 (PEG 4000 - PubChem CID: 174) manufactured by Panreac AppliChem (Darmstadt) in Germany, batch A 545663

and batch B 708221. Kolliphor® P 407 (Poloxamer 407 - PubChem CID: 24751) manufactured by Sigma-Aldrich (St. Louis) in USA, batch A: BCBN0739V, batch B: BCBQ1330V, percentage polyoxyethylene of 71.5-74.9%. Sodium Hyaluronate (PumChem CID: 53447380) distributed by Fagron Iberica S.A.U in Spain, batch L15070231 – OF-06185. Chondroitin Sulphate Sodium (PubChem CID: 24766) donated by SDM (Barcelona) Spain. Cremophor RH 40 distributed by Fagron Iberica S.A.U in Spain, batch L15080090 – OF – 206712. Polyvinyl alcohol (Polyvinyl alcohol - PubChem CID: 11199) manufactured by Panreac AppliChem (Castellar del Vallès, Barcelona) Spain, batch SP013900.

2.2. Methods

Aqueous solutions ranging from 0.2% to 20% (weight/weight) depending on the polymer, were prepared by mass on a Sartorius ENTRIS224-1S analytical balance (Sartorius AG, Goettingen, Germany). All the mixtures were put under agitation with a magnetic stirrer until complete dissolution. All solutions were prepared using reverse osmosis purified water.

The osmolality of all solutions was measured just after their preparation (time zero) and after 15 and 30 days of storage with an Advanced Model 3320 Micro-Osmometer (Advanced Instruments, Inc., Norwood, MA).

The device directly reports osmolality from the determination of the concentration of solutions by means of freezing-point measurements.

A high-precision thermister senses the sample temperature, controls the degree of supercooling and freezes induction, measuring the freezing point of the sample.

The sample is supercooled several degrees below its freezing point where it is in an unstable state, then, by mechanical agitation it's partially crystalized forming an ice water mixture. Immediately a liquid/solid equilibrium is maintained when the liberated heat of fusion causes a rise in the temperature of the sample toward the plateau temperature. The osmometer measures this plateau temperature and calculates the concentration in mOsm/Kg of water (Advanced Instruments. INC, 2015).

Storage conditions were control by using a Memmert climatic chamber (Memmert GmbH + Co. KG, Schwabach, German), with a control temperature of 40 ± 3 ° C.

For the statistical analysis, STATGRAPHICS Plus for Windows 5.1. Professional Edition 1994–2000 from Statpoint Technologies, Inc., 560 Broadview Ave, Suite 201, Warrenton, VA 20186 was used. Multifactor analysis of variance, linearization (when needed) and multiple linear regression were performed for all samples to determine the best predictive equation.

3. Results and discussion

The need for experimental determination of osmolality has been established and usually determined, by measuring the freezing point depression (Remington, Joseph P. (Joseph Price) and Beringer, Paul, 2006). Theoretically, osmolality can be calculated from the value of any colligative property of the solution (The United States Pharmacopeial Convention, 2017), but there are not always known and their determination is not regularly affordable in the pharmaceutical development field.

The dissolution of a solute in a pure solvent induces changes in a direct proportion to the solute concentration in the solution´s colligative property (Advanced Instruments. INC, 2015)

- the freezing point is depressed,
- boiling point is raised,
- osmotic pressure is increased, and
- vapor pressure is lowered.

Determining the concentration of a nonvolatile solute in an aqueous solution can be easy and precise by measuring its freezing point depression.

The change in the freezing point of a pure solvent when a solute is dissolved is directly proportional to the molar concentration of the solute and can be determined using the following equation:

$$\Delta T = K_f \cdot m$$

Equation 1.1. Freezing point depression

Where Δt is the temperature change from the pure solvent's freezing point to the freezing point of the solution, K_f is the freeing point depression constant (for water is 1.86 kg°C /mol) and·m is the molal concentration of the nonvolatile solute (Chang, 2007).

Freezing point measurement cannot tell us what the nature of the particles is (size, shape or conformation); as it only depends on the number of particles in solution.

For practical purposes, osmolarity (a theoretical quantity) expressed in osmoles per L of a solution is widely used (The United States Pharmacopeial Convention, 2017) and calculated from the w / v concentration using the Equation 1.2. (Remington, Joseph P. (Joseph Price) and Beringer, Paul, 2006).

$$Osmolarity\ \left(\frac{mOsm}{L}\right) = Concentration\ \left(\frac{g}{L}\right) \times \frac{1}{Molelcular\ weight}\left(\frac{mol}{g}\right) \times\ number\ osmol/mol\ \times \frac{1000mOsm}{Osm}$$

Equation 1.2. Theoretical osmolality

The number of osmol/mol is equal to 1 for nonelectrolytes and is equal to the number of ions per molecule for strong electrolytes. This calculation assumed an ideal solution and omits factors such as solvation and interionic forces.

To compute osmolality from values of theoretical osmolarity (obtained from equation 1.2), a density of 0.997 g/ml was used (Thiesen et al., 1900).

When the osmolality of aqueous solutions of different polymers was determined theoretically, the values obtained were different from those obtained by the measurement of the freezing point depression. In pre-formulation, this can lead to the selection of an unsuitable polymer or its concentration and cause errors in the formulation processes lost in time and resources.

These differences were expected as the osmolalities of single-solute solutions are represented in the Osmotic Virial Equation (OVE) as polynomials in concentration, where each solute has coefficients for terms of second or higher order in concentration (Elliott et al., 2007; Prickett et al., 2011), also, interactions between solutes must be taken into account.

Osmolality of polymers, depend more on the degree of polymerization and the interaction between the chains, than the number of active particles in solution (molecules or ions) (Cloizeaux, 1975; Zavitsas, 2001).

3.1. Polyethylene glycol 400 (PEG400)

PEG 400 is widely used in different pharmaceutical formulations, in parenteral, topical, ophthalmic, oral, and rectal preparations (Martindale, 2016a). As far as ophthalmic and parenteral preparations are concerned, the addition of PEG 400 caused an increase in/mol the osmolality, due to its action as a hyperosmolar agent.

A multifactor analysis of variance for the osmolality of different aqueous PEG 400 solutions was performed to determine if the concentration, batch and/or the time have a statistically significant effect on the osmolality.

Due to its intrinsic properties and as seen in graphic 1, the theoretical osmolality is exhibits quite a difference from the experimental osmolality, especially at high concentrations. This effect, observed by other authors (Money, 1989), can be explained by a possible change in the PEG`s molecule configuration in response to the change in the concentration; going from extended to folded configuration when increasing the concentration. This folded configuration exposed oxygens that bind with the hydrogens of the water, depressing the thermodynamic activity of water and increasing the osmolality.

In a multifactor analysis of the variance for the PEG's 400 osmolality, the p-value for concentration, time and the interaction between these two factors (AB) were less than 0.05, indicating that there is a statistically significant effect on the PEG's 400 osmolality at the 95 % confidence level for all three factors.

Graphic 1 shows a strong relation between the osmolality and the PEG 400 concentration and time of storage at 40 ºC.

(Graphic 1)

To linearized the data obtained, a square root for PEG's 400 osmolality was applied due to the quadratic relation showed in the results obtained from the variance analysis with polynomic contrast (F-value 152, p-value < 0.01) and explained in Graphic 2.A.

(Graphic 2)

To simplify the model, a multiple linear regression using the backward selection method was applied to remove from the equation the factor(s) with the highest p-value and no significance: as a result, the time of storage was removed. As seen in Graphic 2, the simplified equation fitted the model and explains 96.9 % of the variability in the square root of the osmolality.

$$\sqrt[2]{\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right)} = 4.14 + (0.15 * PEG400\left(\frac{\text{g}}{\text{kg}}\right)$$

Equation 2. Predicted osmolality of PEG 400 solutions

Equation 2 can be used as a tool for predicting the osmolality for PEG 400`s aqueous solutions in the range of the studied concentrations and storage conditions.

3.2. Polyethylene glycol 4000 (PEG4000)

Like other polyols, PEG 4000 caused an increase in the osmolality, as it acts as a hyperosmolar agent.

Graphic 3 shows the behaviour of the osmolality for the two different batches, A and B, at different concentrations and time of storage at 40 ºC.

(Graphic 3)

The multifactor analysis of the variance for the PEG's 4000 osmolality indicated that all three factors and the interaction between them had statistically significant effect on the osmolality (p-value < 0.05). On the other hand, the polynomic contrast (F-value 1896 p-value < 0.01) indicated a quadratic increasing tendency of the osmolality in behalf of the concentration.

(Graphic 4)

The backward selection method excluded the batch variable. Graphic 4 shows the representation of the fitted model which explains 99.2% of the variability in the square root of osmolality.

The equation of the fitted model is Equation 3

$$\sqrt[2]{\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right)} = -0.5 + \left(0.12 * PEG4000\left(\frac{\text{g}}{\text{kg}}\right)\right) + \left(0.02 * time(days)\right)$$

Equation 3. Predicted osmolality of PEG 4000 solutions

Equation 3 can be used as a tool for predicting the osmolality for PEG 4000`s aqueous solutions in the range of the studied concentrations and storage conditions.

3.3. Poloxamer 407

Poloxamers are synthetic block copolymers of ethylene oxide and propylene oxide, widely used in cosmetics and pharmaceuticals (Arbelaez-Camargo et al., 2016). They are used as emulsifying agents for intravenous fat emulsions, as solubilising agents to maintain clarity in elixirs and syrups, and as wetting agents for antibacterials (Paul J Sheskey, 2015b).

Like other poloxamers, Poloxamer 407`s (P407) osmolality was expected to be insignificant in aqueous solutions because of its high molecular weight (12,200 g/moles), but this was not the case. As observed by other authors, the osmolality was described by polynomial equations (Viegas and Henry, 1998).

The multifactor analysis of the variance for the P407 osmolality indicated that all three factors studied and the interaction between them had statistically significant effect on the osmolality (p-value < 0.05). As shown in Graphic 5, a quadratic increasing tendency of the osmolality was indicated by polynomic contrast (F-value 203 p-value < 0.01)..

(Graphic 5)

Graphic 5 shows that as the concentration of P407 increases, the osmolality increases in a non-linear way. At low concentration where the gel is not form, the curve is linear; when increasing the concentration, solvent-polymer interactions and intermolecular associations become stronger causing a loss of linearity (Viegas and Henry, 1998). The so that is bound to the polymer should be considered part of the solute and not part of the dissolving solvent. Water molecules bound to the solute more strongly than to other water molecules, making easier the elimination of water in the freezing point depression (Zavitsas, 2001). As the

temperature increased, the same behaviour occurred and as a result, the osmolality increased when stored at 40 ºC for 15 and 30 days, but in a lesser measure (Equation 4).

(Graphic 6)

Graphic 6 shows the representation of the fitted model that explains the 94 % of the variability in the square root of osmolality in the studied conditions, taking into account that the backward selection method excluded the batch variable.

The equation of the fitted model represented in Graphic 6.A,

$$\sqrt[2]{\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right)} = -0.8 + \left(0.11 * P407\left(\frac{\text{g}}{\text{kg}}\right)\right) + \left(0.03 * time(days)\right)$$

Equation 4 Predicted osmolality of P407 solutions

Equation 4 can be used as a tool for predicting the osmolality of P407`s aqueous solutions in the range of the studied concentrations and storage conditions.

3.4. Sodium Hyaluronate (SH)

Hyaluronic acid is a natural polymer commonly distributed in the body. It is part of different body tissues and intracellular fluids as the vitreous humour and synovial fluid (Martindale, 2016b). Sodium hyaluronate (SH) is the predominant form of hyaluronic acid at physiological pH and is the most common form used in cosmetics, parenteral, and ophthalmic pharmaceutical formulations ("Sodium Hyaluronate: Pharmaceutical Excipients," n.d.). Since SH is widely used as an excipient in different pharmaceutical forms and as active principle for intra-articular treatment in knee osteoarthritis (Altman et al., 2015; Duarte et al., 2012; Hoare and Kohane, 2008; Ito et al., 2007; Mayol et al., 2008), knowledge of it behaviour in terms of physicochemical properties, such as the osmolality, is of great interest to predict it behaviour in the pharmaceutical development. With an average molecular weight of 12,220 g/moles and the formation of large size aggregates in solution, it was excepted to have a negligent osmotic effect. Graphic 7 shows the behaviour of SH aqueous solutions at different concentrations and times of storage at 40 ºC. As shown, the osmolality determined experimentally differed from the theoretical value.

(Graphic 7)

The study of the variability of the osmolality of the SH aqueous solutions due to the concentration and time of storage at 40 ºC was statistically analyzed. At a confidence level of 95%, it can be stated that only the concentration has a statistically significant effect in the osmolality (p-value < 0.05) on the studied conditions.

The SH properties are the results of the properties of a population of molecules with a wide chain length, as is established to be polydispersed. In solution, it behaves as a stiffened random coil that occupies a large hydrated volume and consequently has solute–solute interactions at an unusually low concentration (Hardingham, 2004).

The osmolality corresponds to the concentration of osmotically active molecules, capable of exerting osmotic pressure. SH, a biopolymer with multiple roles in almost all biological tissues, has a high osmotic pressure that reinforces various physicochemical properties necessary to maintain the hydration and lubrication of tissues. One of the factors contributing

to the high observed osmolalities, is the polyelectrolytic character of the molecule due to dissociation of the counter- ion (Horkay et al., 2009).

(Graphic 8)

As shown in Graphic 8, a squared-SH concentration model described 96.3% of the relationship between the osmolality and the SH concentration. Equation 5 explained the fitted model.

$$\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right) = 0.60 + 0.23 * SH\left(\frac{\text{g}}{\text{kg}}\right)^2$$

Equation 5. Osmolality prediction for SH solutions

The correlation coefficient equal 0.98, indicating a relatively strong relationship between the variables.

Equation 5 can be used as a tool for predicting the osmolality for HS`s aqueous solutions in the range of the studied concentrations and storage conditions

3.5. Chondroitin Sulphate Sodium (CS)

Chondroitin sulphate is a natural polymer present in most cartilaginous tissues. For medical applications, the sodium salt, chondroitin sodium sulphate, is used orally and in ocular surgical procedures, among others (Martindale, 2016d).

(Graphic 9)

As expected for a highly charged molecule, CS`s associated counter-ions generate an osmotic pressure, represented by the osmolality, that plays an important biologic effect in tissue hydration. The theoretical osmolality calculated from the Equation 2, considers the contribution of the counter-ions in the osmolality, thus, the theoretical osmolality is far from the observed osmolality (Graphic 9). These results reinforce the theory, that even though the electrostatic contribution is of great importance, it cannot be taken into account by itself, since the non-electrostatic contributions coming from the intermolecular and solute interactions also affect the osmolality, particularly at low concentrations in which non-electrostatic contributions account for about 1/3 of the total osmotic pressure (Bathe et al., 2005; Ehrlich et al., 1999). It is known that at low temperatures CS is stable, but in the other hand, at high temperatures and after 500-600 hours, degradation process occurs producing low-molecular-mass fragments and desulfated products (Volpi et al., 1999), that will contribute, increasing the osmolality.

The influence of the CS concentration and the time of storage at 40 ºC on the osmolality was evaluated at a confidence level of 95%. The results showed that the concentration, the time of storage at 40 °C and their interaction had a statistically significant effect in the osmolality (p-value <0.05) on the studied conditions.

A multiple linear regression using the backward selection method was applied to simplify the model, the result excluded the time of storage, but included the interaction between it and the concentration. The hierarchical principle indicates that if a model contains the interaction between two variables it must take into account both variables, whereby, the simplified model was not used (Bishop et al., 2007).

The fitted linear regression model shown in Graphic 9, explained 94.8 % of the variability in osmolality at a 95 % confidence level.

(Graphic 10)

Equation 6 of the fitted model is represented in Graphic 10.A

$$\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right) = -0.67 + \left(1.87 * CS\left(\frac{\text{g}}{\text{kg}}\right)\right) + \left(0.01 * time(days)\right) + \left(0.01 * \left(CS\left(\frac{\text{g}}{\text{kg}}\right) * time(days)\right)\right)$$

Equation 6 Predicted osmolality of CS solutions

Equation 6 can be used as a tool for predicting the osmolality for CS`s aqueous solutions in the range of the studied concentrations and storage conditions

3.6. Cremophor RH 40

The main constituent of Cremophor RH 40 (CRE 40) is glycerol polyethylene glycol hydroxy- stearate, which, together with fatty acid glycerol polyglycol esters, forms the hydrophobic part of the product. Despite its surface active properties, CRE does not cause haemolysis and its use in pharmaceutical preparations for parenteral application (BASF SE Care Chemicals Division Pharma Ingredients & Services, 2008).

Graphic 11 shows a deviation from the theoretical osmolality at concentrations higher than 100 g/kg. This effect may be due to the molecular behaviour of the CRE 40 in solution but insufficient information is available to explain the deviation observed.

(Graphic 11)

The maximum CRE 40 concentration studied had an osmolality below the physiological osmolality, which may explain the fact that it does not cause hemolysis despite its surface active properties (Hork and Lais, 2002).

The statistical study of the osmolality of Cremophor Polyoxyl 40 Hydrogenated Castor Oil evaluated at a confidence level of 95%, showed that both, the concentration and the time of storage at 40 °C, had statistically significant effect on the osmolality (p-value < 0.05).

A multiple linear regression using the backward selection method was applied to simplify the model and excluded the storage time and the interaction between the storage time and concentration.

(Graphic 12)

The fitted model expressed by Equation 7 and shown in Graphic 12, explains 99.5% of the variability in the osmolality. at a 95 % confidence level.

$$\sqrt[2]{\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right)} = 0.18 + \left(0.07 * CRE\ 40\left(\frac{\text{g}}{\text{kg}}\right)\right)$$

Equation 7 Predicted osmolality of CRE 40 solutions

Equation 7 can be used as a tool for predicting the osmolality for CCRE 40`s aqueous solutions in the range of the studied concentrations and storage conditions

3.7. Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a polar polymer widely used because of its mechanical properties, high melting temperature and biocompatibility (Tacx et al., 2000). It is used both alone and crosslinked. It is also used in the formulation of hydrogels, nanoparticles and beads.

With a molecular weight of approximately 85000 g/mol, it is expected to have a negligible osmolality. The results obtained, as observed in the Graphic 13, showed an increase of the osmolality when increasing the concentration. A slight increase in osmolality due to time in the conditions studied was also observed.

(Graphic 13)

Statistical multifactor analysis of the osmolality indicated that both, the concentration and storage time at 40 ºC, had a statistically significant effect on the osmolality (p-value < 0.05), but their interaction did not. This effect can be explained by the fact that PVA, in water, forms an intricate molecular network in which there is water of hydration bound to the polymer-free hydroxyl groups acting like a zipper between two polymer chains. This results in the formation of aggregates, decreasing the free water in the solution. At high temperatures there is a higher interaction between water and PVA molecules, breaking the zipper action; undoing aggregates; and facilitating the interaction of the PVA free chains with water-free molecules: decreasing free water molecules (Krzeminski and Molisak-tolwinska, 1991; Tacx et al., 2000; Vigneswari et al., 2016).

A multiple linear regression using the backward selection method was applied to simplify the model, the result excluded the time of storage at 40 ºC and explained the 96.0 % of the variability in osmolality.

(Graphic 14)

The equation of the fitted model is:

$$\sqrt[2]{\text{Osmolality}\left(\frac{\text{mOsm}}{\text{kg}}\right)} = 2.0 + \left(0.09 * PVA\left(\frac{\text{g}}{\text{kg}}\right)\right)$$

Equation 8. Predicted osmolality of PVA solutions

Equation 8 can be used as a tool for predicting the osmolality for PVA`s aqueous solutions in the range of the studied concentrations and storage conditions

4. Conclusions

The data obtained from this study showed that the theoretical determination of the osmolality of the different polymers; based on molecular weight as the only determinant factor, did not agree with the values obtained by measurement of colligative properties, such as the freezing point depression. The data analysis provided an understanding of the osmolality behaviour of the different studied polymers in aqueous solution and its evolution over a period at the hypothetical worst case of storage temperature. Application of this knowledge would facilitate primary galenic development of safe parenteral and ophthalmic dosage forms in terms of osmolality, by its estimation using the predictive obtained equations.

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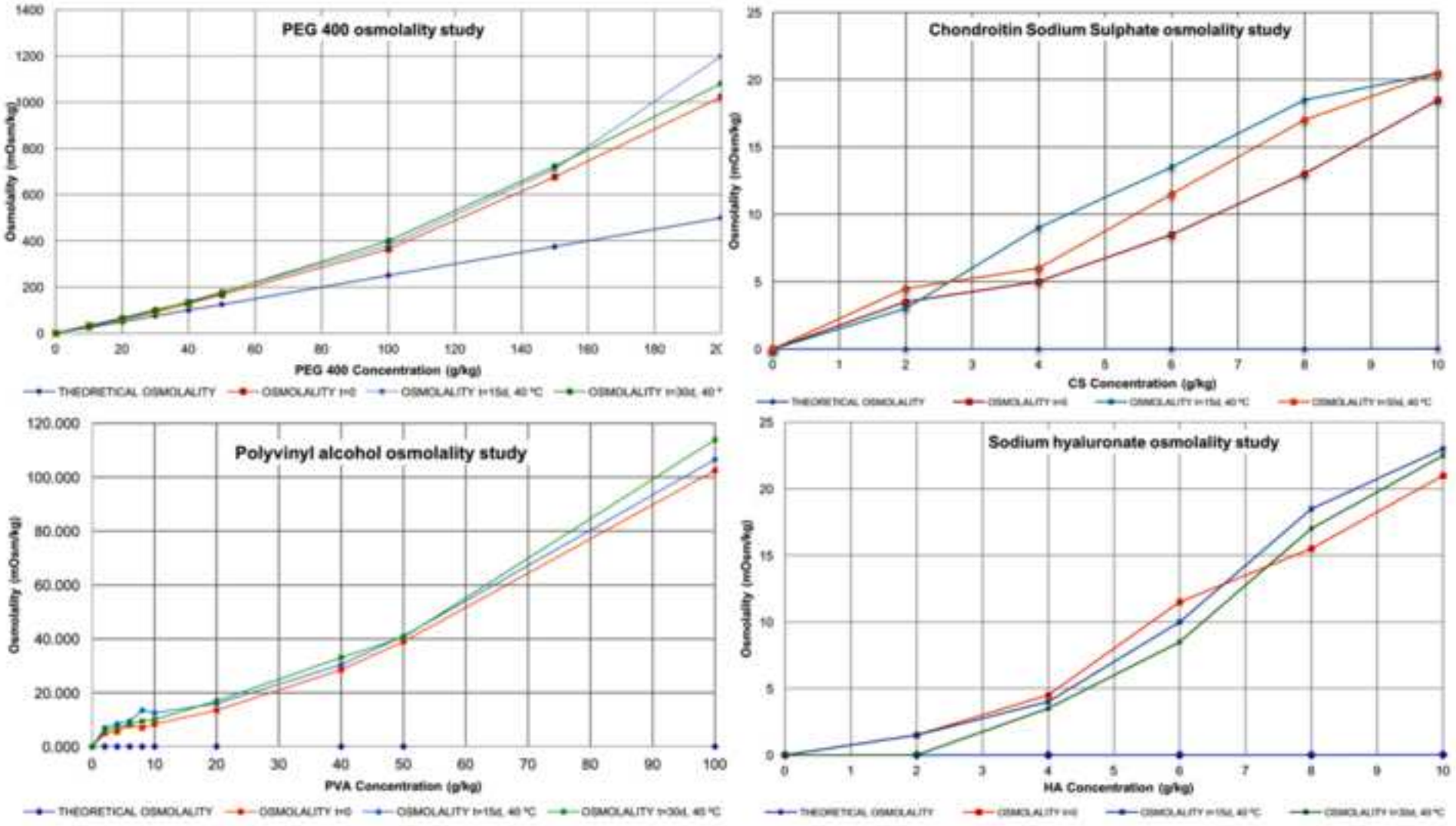
*Graphical Abstract (for review)



Osmolality predictive models of different Polymers as tools in parenteral and ophthalmic formulation development

***Response to Reviewers**

Reviewers' comments:

Reviewer #1:

The authors thank the reviewer for the comments. Sure, they will improve the quality of the manuscript.

Specific comments:

Major:

1) More details on the applied mathematical equation used for calculating the theoretical osmotic pressure must be given. Also, more details on the determination of the freezing point depression must be given.

We agree that there is a lack of information on the determination of osmolality by freezing point depression. More details are given, now, in point 2.2 Methods, line 100 -110.

2) The units in Equation 1 are confusing.

Equation 1: each term in brackets has been explained following the equation.

3) The English needs to be improved throughout the manuscript.

A review of the entire text has been made.

4) All figures concerning a particular polymer should be combined (in Figure a, b, c).

5) The figure legends must be more detailed.

6) The figures should be more carefully be prepared, e.g. often the number of indicated digits is unnecessarily high. Also, some of the text is overlapping.

7) The color code should be uniform throughout the paper.

Some of the figures can´t be combined, since there would be an overlap between batches and the evolution in time would not be clear. But, in this way all figures, were corrected in terms of number of digits, colors and possible overlapping.

Minor:

8) Not textbooks should be cited for the use of the polymers, but recent review articles.

References Mathew et al., 2017; Nguyen et al., 2015; Sivashanmugam et al., 2015; Thambi et al., 2017 are addeed.

Reviewer #2: This is an interesting article reporting measurements of osmolality of various polymeric and oligomeric materials used in pharmaceutical formulations. The measured data are compared to 'theory' and in general the data are not predicted well by the theory. Empirical linear regressions are determined for calculating the osmolality based on concentration and time in storage. The data and regressions appear to be done correctly to me, but there are some missing details that would make this paper more appealing to a broader audience. However, the description of the theory is not sufficient and needs to be revised. Some detailed comments and questions about this are given below.

The authors thank the reviewer for the comments. Sure, they will improve the quality of the manuscript.

1) Equation (1) is not clear about what each term in brackets represents. Right now they are simply units and readers versed in this field can parse out what they mean. However, to be done properly it would be best if the equation used variables or text to precisely describe each quantity being multiplied on the right hand side (concentration, molecular weight, etc.). Eq. strictly refers to

osmolarity, while the data present osmolality (although I realize that in practice these are very close numerically). The concentration in Equation (1) is molarity, while the concentration used in all of the plots is a weight fraction. I assume - but it should be stated in the paper - that the authors have used some value of water density to convert these. Also, equation (1) is certainly not the only theory available to predict osmolarity, but is the most common and based on ideal solution assumption where osmolarity is assumed equal to concentration of solute particles.

Equation 1: each term in brackets has been explained. We add in line 155, the density used to convert osmolarity to osmolality is clarified.

2) The freezing point depression instrument: does it directly report osmolality, or have the authors calculated osmolality from the freezing point depressions themselves? In either case, how is the temperature depression converted into an osmolality? Most likely this is also using an ideal solution model, but it would be nice to be clarified.

We agree that there is a lack of information on the determination of osmolality by freezing point depression. Additional details are given in point 2.2 Methods, line 100 -110.

3) There is a statement in the theory section about osmotic pressure on of polymers being more of a function of molecular weight and interactions than on number of particles. However, osmotic pressure per se is not reported in this paper. It seems to be assumed however that the reader will know that osmotic pressure is in the same class of property as freezing point depression - is this a reasonable assumption for the readership?

We agree that the statement can be confusing to readers, that is why we have modified the statement in line 165.

4) It would be useful to point out for readers that a theoretical basis exists for expecting a quadratic dependence on concentration (related to point (3)). Physical chemistry or polymer science texts should describe so-called virial expansions where 2nd-order (quadratic) terms in concentration (c^2) are added to the ideal solution theory (equation 1) to correct for solute interactions.

In point 3. Result and Discussion, from line 125 to 165, more theoretical basis of the ideal solution model and the virial expansions were made.

5) What is the water source used to prepare the solutions? Is it distilled, deionized or filtered in some way?

In point 2.2 Methods, line 100 -110. Also, in this section we clarified the type of water used in the preparation of the samples

6) What is the expected significance to pharmaceutical formulators that the ideal solution model badly predicts osmolality of these commonly used polymers?

A clearer explanation is given in line 157.

Figures:

Graphic 1. Experimental osmolalities of differently concentrated aqueous solutions of PEG 400`s after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of PEG 400, estimated using Equation 1.2.

Graphic 2. (A) PEG 400's osmolality square root for linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.

Graphic 3. Experimental osmolalities of differently concentrated aqueous solutions of PEG 4000`s and two different product batches, just after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of PEG 4000's, estimated using Equation 1.2.

Graphic 4. (A) PEG 4000's square root for osmolality linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.

Graphic 5. Experimental osmolalities of P407`s aqueous solutions at different concentrations and two different batches, just after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of P407's, estimated using Equation 1.2.

Graphic 6. (A) P407's osmolality square root for linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.

Graphic 7. Experimental osmolalities of differently concentrated aqueous solutions of SH's after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of SH's, estimated using Equation 1.2.

Graphic 8. (A) Squaring SH concentration for linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.

Graphic 9. Experimental osmolalities of differently concentrated aqueous solutions of CS`s after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of CS, estimated using Equation 1.2.

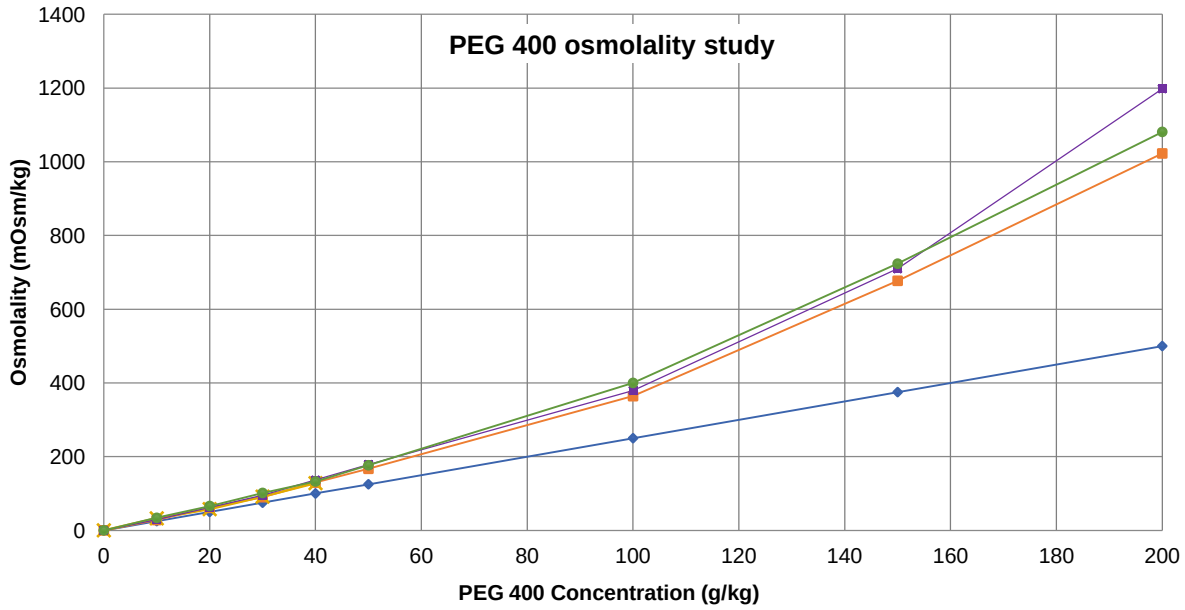
Graphic 10. (A) Linear regression to describe the original and the fitted model of the Osmolality vs CS's concentration plot. (B) Observed vs Predicted Scatter Plot.

Graphic 11. Experimental osmolalities of differently concentrated aqueous solutions of CRE 40`s after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of CRE 40, estimated using Equation 1.2.

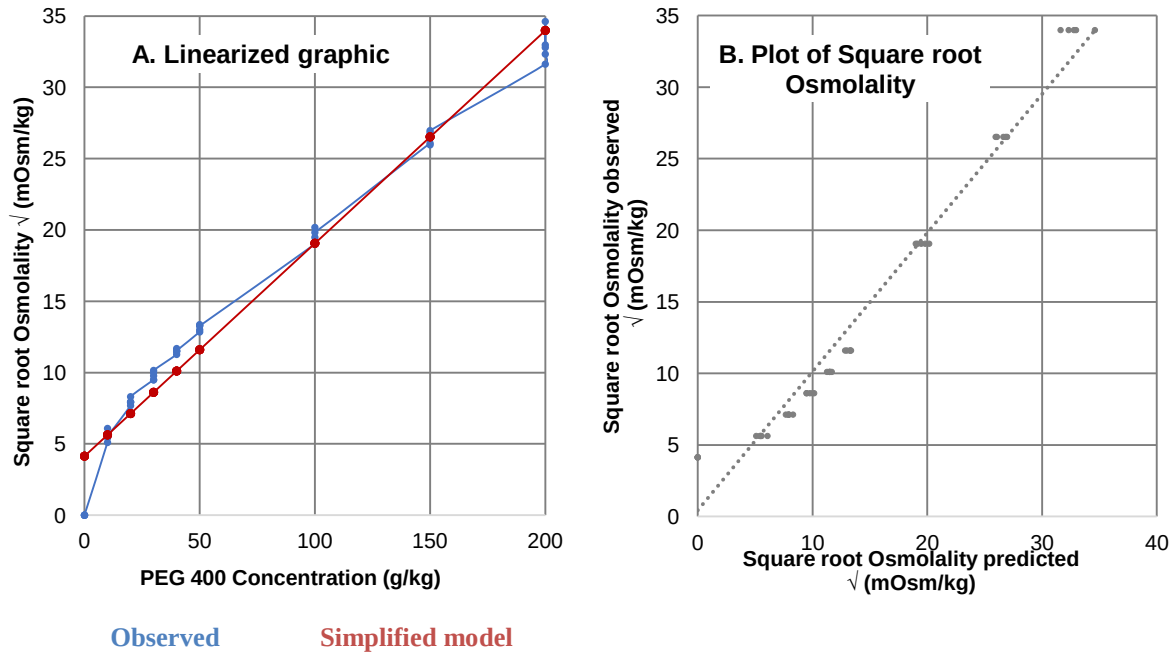
Graphic 12. (A) CRE 40's osmolality square root for linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.

Graphic 13. Experimental osmolalities of differently concentrated aqueous solutions of PVA's after their preparation (time zero) and after 15 and 30 days of storage. Theoretical osmolalities of differently concentrated aqueous solutions of PVA, estimated using Equation 1.2.

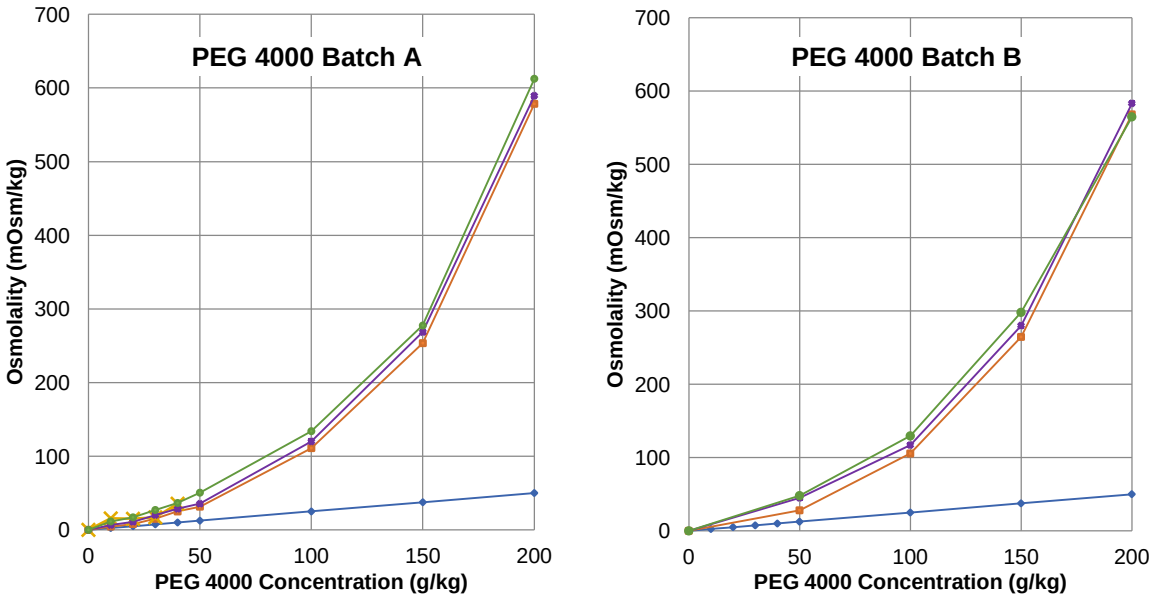
Graphic 14. (A) PVA's osmolality square root for linearization to describe the original nonlinear model and the fitted model. (B) Observed vs Predicted Scatter Plot.


PEG 400 osmolality study
Osmolality (mOsm/kg)
1400
1200
1000
800
600
400
200
0
0
20
40
60
80
100
120
140
160
180
200
PEG 400 Concentration (g/kg)
Theoretical osmolality
Osmolality t=0
Osmolality t=15 d, 40 ºC
Osmolality t=30 d, 40 ºC

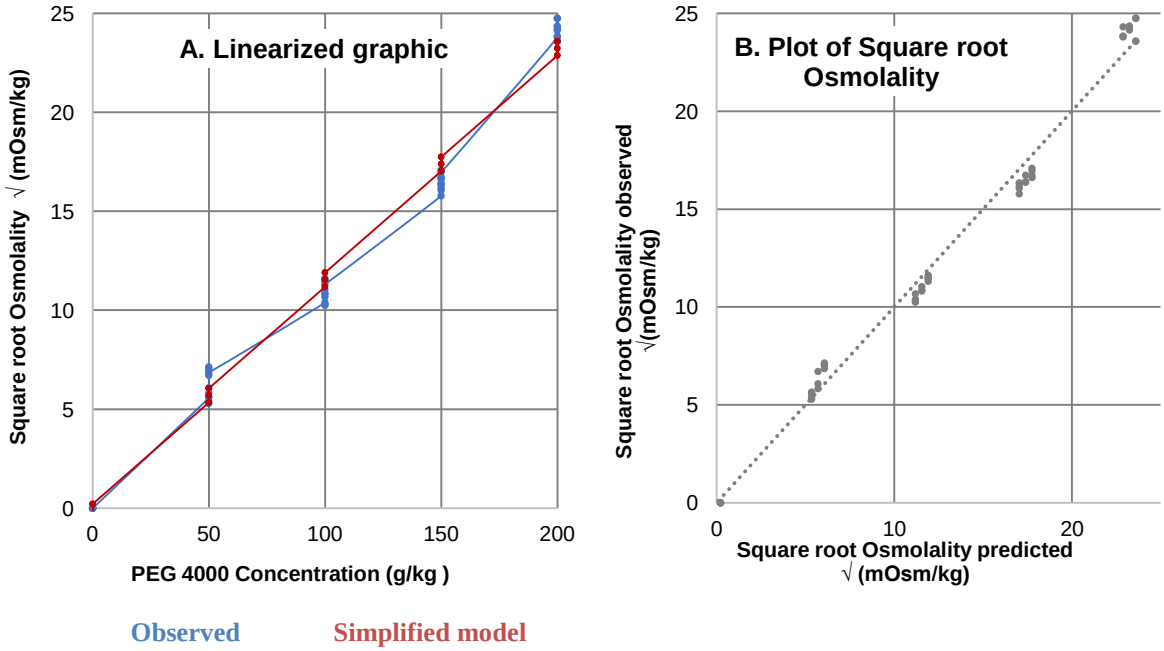
Graphic 1


A. Linearized graphic
Square root Osmolality √ (mOsm/kg)
PEG 400 Concentration (g/kg)
0
5
10
15
20
25
30
35
0
50
100
150
200
B. Plot of Square root Osmolality
Square root Osmolality observed √ (mOsm/kg)
Square root Osmolality predicted √ (mOsm/kg)
0
10
20
30
40
Observed
Simplified model

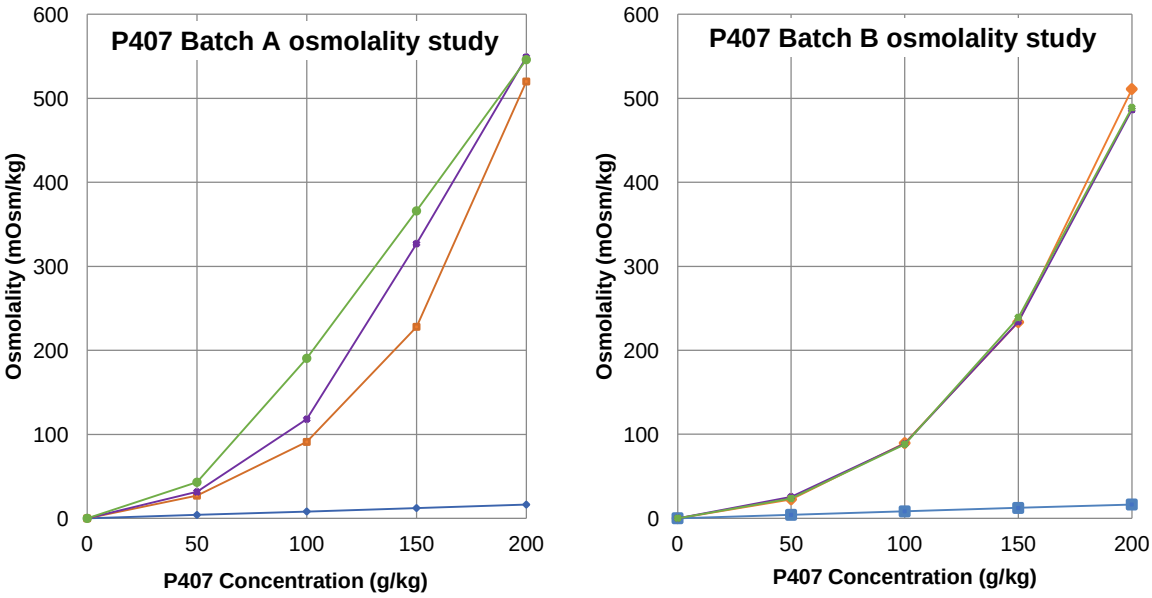
Graphic 2


PEG 4000 Batch A
Osmolality (mOsm/kg)
PEG 4000 Concentration (g/kg)
PEG 4000 Batch B
Osmolality (mOsm/kg)
PEG 4000 Concentration (g/kg)
Theoretical osmolality
Osmolality t=0
Osmolality t=15 d, 40 ºC
Osmolality t=30 d, 40 ºC

Graphic 3

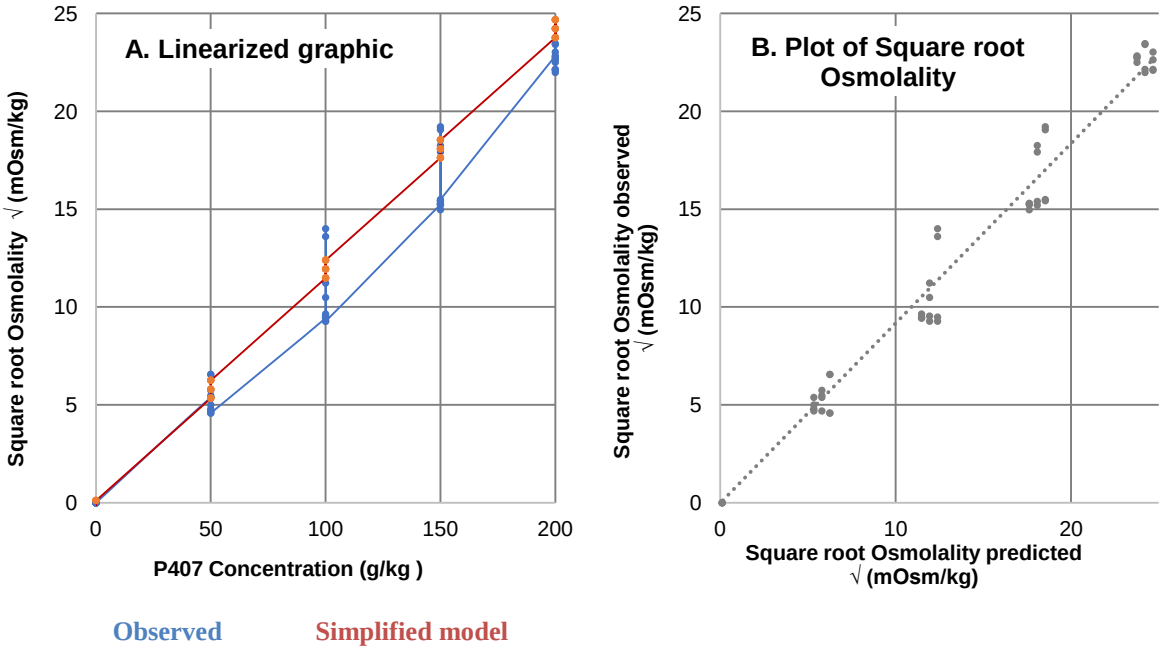

A. Linearized graphic
Square root Osmolality √ (mOsm/kg)
PEG 4000 Concentration (g/kg)
Observed
Simplified model
B. Plot of Square root Osmolality
Square root Osmolality observed √(mOsm/kg)
Square root Osmolality predicted √ (mOsm/kg)

Graphic 4

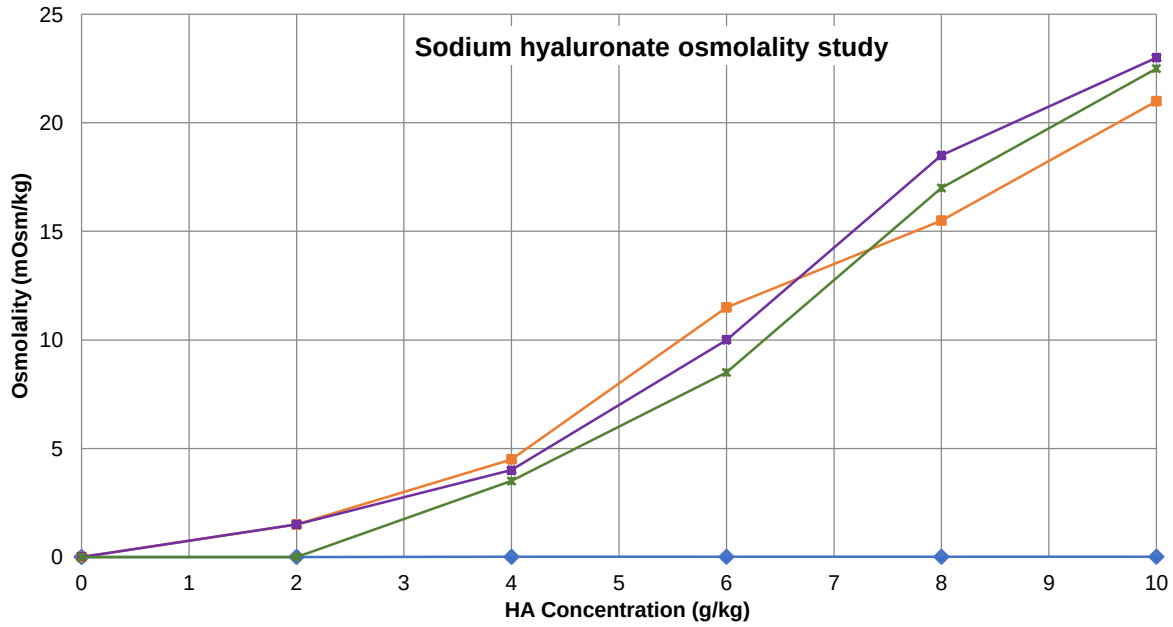


Theoretical osmolality **Osmolality t=0** **Osmolality t=15 d, 40 ºC** **Osmolality t=30 d, 40 ºC**

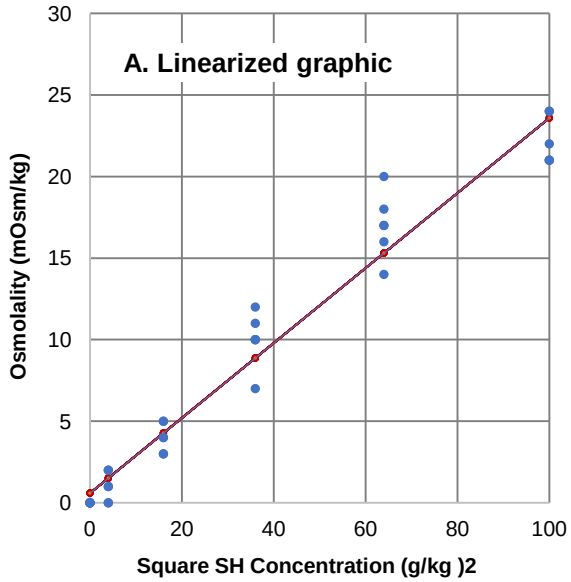
Graphic 5

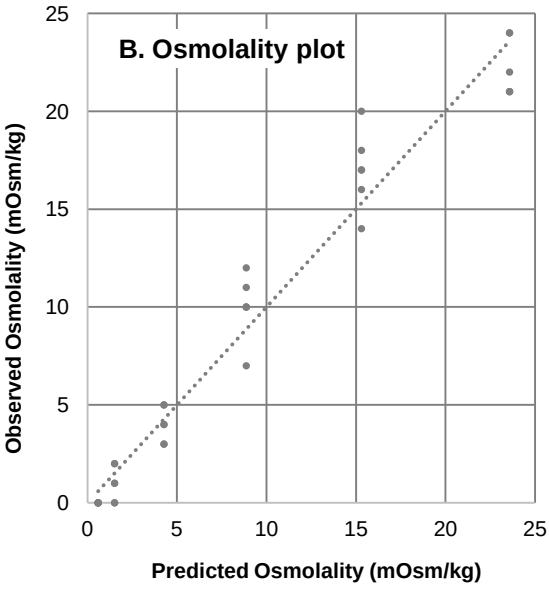

A. Linearized graphic
Square root Osmolality √ (mOsm/kg)
P407 Concentration (g/kg)
0
5
10
15
20
25
0
50
100
150
200
B. Plot of Square root Osmolality
Square root Osmolality observed √ (mOsm/kg)
Square root Osmolality predicted √ (mOsm/kg)
0
10
20
Observed
Simplified model

Graphic 6.


Sodium hyaluronate osmolality study
Osmolality (mOsm/kg)
HA Concentration (g/kg)
0
5
10
15
20
25
0
1
2
3
4
5
6
7
8
9
10
Theoretical osmolality
Osmolality t=0
Osmolality t=15 d, 40 ºC
Osmolality t=30 d, 40 ºC

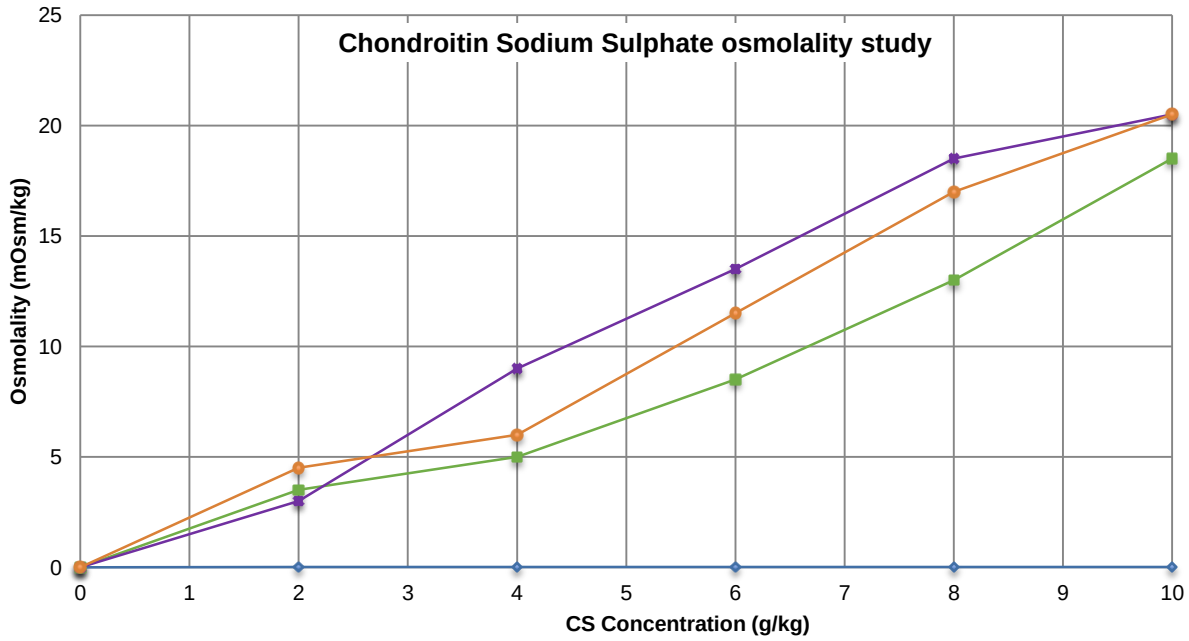
Graphic 7



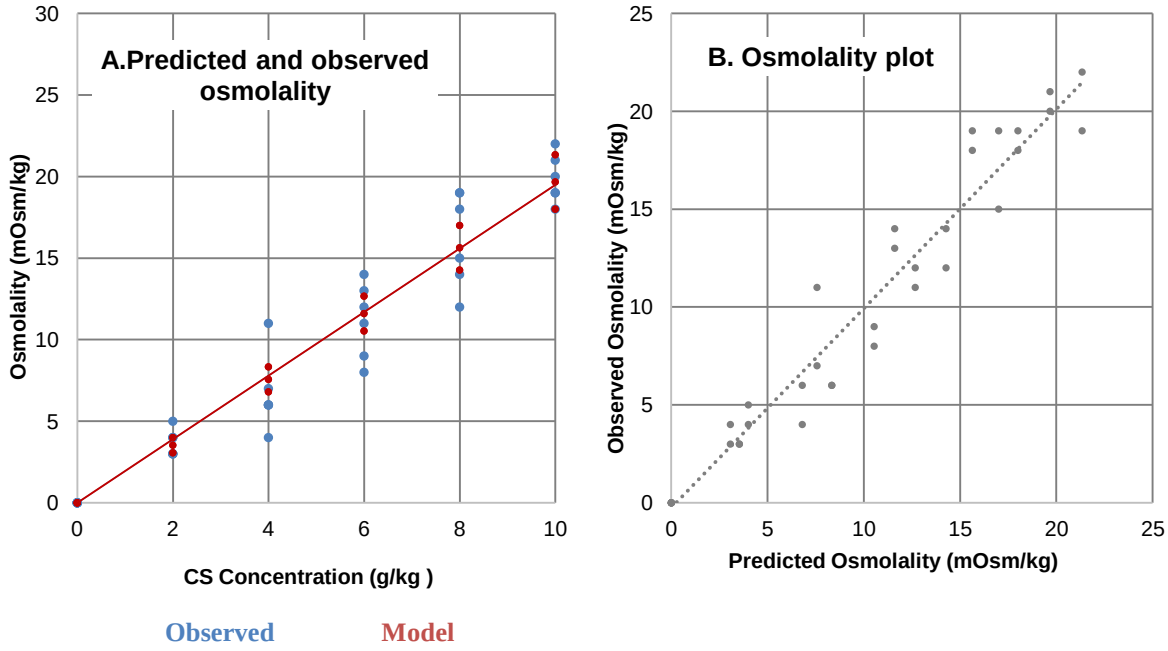


Observed Model

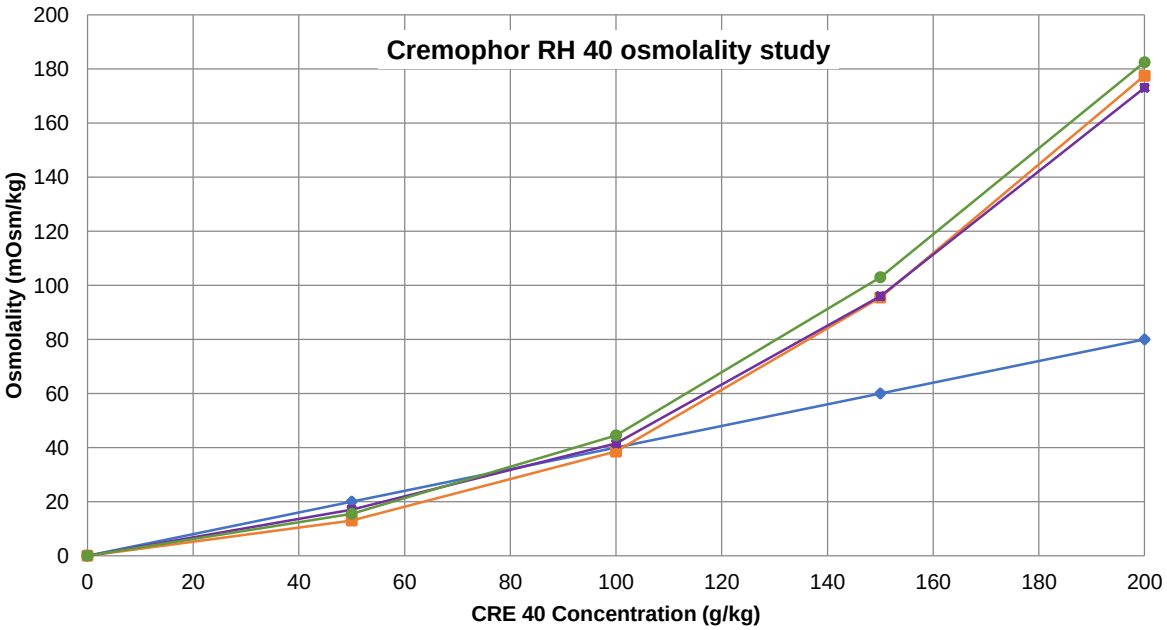
Graphic 8


Chondroitin Sodium Sulphate osmolality study
Osmolality (mOsm/kg)
25
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
CS Concentration (g/kg)
Theoretical osmolality
Osmolality t=0
Osmolality t=15 d, 40 ºC
Osmolality t=30 d, 40 ºC

Graphic 9

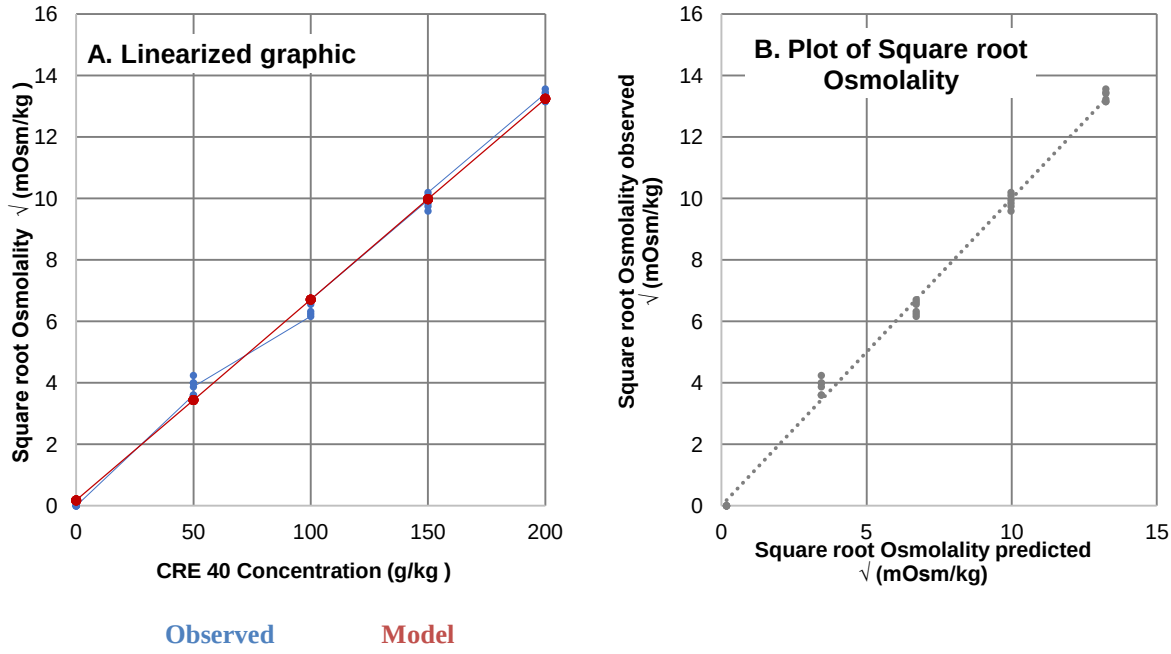

A.Predicted and observed osmolality
Osmolality (mOsm/kg)
CS Concentration (g/kg)
Observed
Model
B. Osmolality plot
Observed Osmolality (mOsm/kg)
Predicted Osmolality (mOsm/kg)

Graphic 10

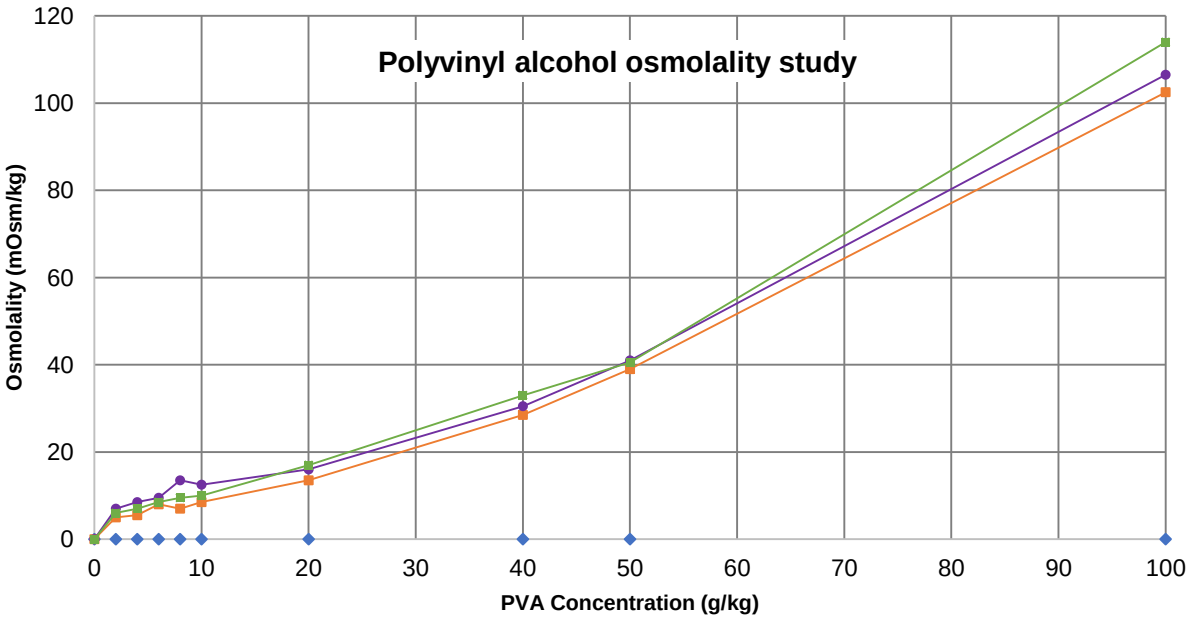


Theoretical osmolality **Osmolality t=0** **Osmolality t=15 d, 40 ºC** **Osmolality t=30 d, 40 ºC**

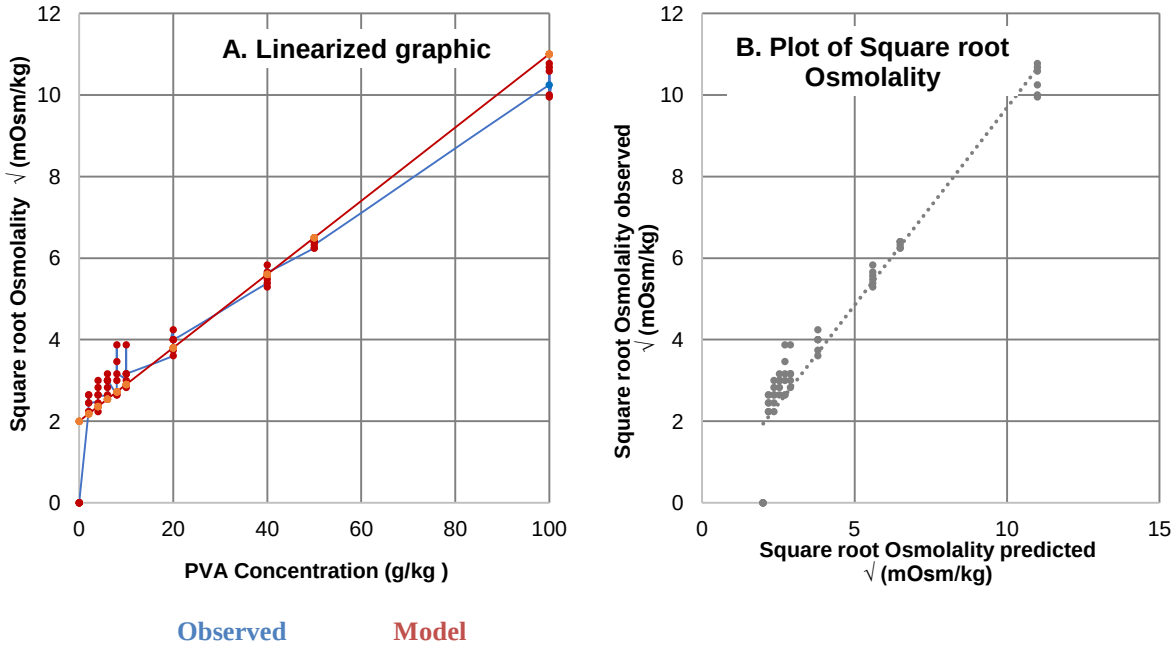
Graphic 11


A. Linearized graphic
Square root Osmolality √ (mOsm/kg)
CRE 40 Concentration (g/kg)
0
2
4
6
8
10
12
14
16
0
50
100
150
200
B. Plot of Square root Osmolality
Square root Osmolality observed √ (mOsm/kg)
Square root Osmolality predicted √ (mOsm/kg)
0
5
10
15
Observed
Model

Graphic 12


Polyvinyl alcohol osmolality study
Osmolality (mOsm/kg)
PVA Concentration (g/kg)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
70
80
90
100
Theoretical osmolality
Osmolality t=0
Osmolality t=15 d, 40 ºC
Osmolality t=30 d, 40 ºC

Graphic 13


A. Linearized graphic
Square root Osmolality √ (mOsm/kg)
PVA Concentration (g/kg)
Observed
Model
B. Plot of Square root Osmolality
Square root Osmolality observed √ (mOsm/kg)
Square root Osmolality predicted √ (mOsm/kg)

Graphic 14.