



## Osmolality of excipients for parenteral formulation measured by freezing point depression and vapor pressure – a comparative analysis

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1 OSMOLALITY OF EXCIPIENTS FOR PARENTERAL  
2 FORMULATION MEASURED BY FREEZING POINT  
3 DEPRESSION AND VAPOR PRESSURE – A COMPARATIVE  
4 ANALYSIS

5  
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## **Abstract**

### **Purpose**

To investigate the difference in methods to determine the osmolality in solutions of stabilizers used for long-acting injectable suspensions.

### **Methods**

The osmolality was measured by freezing point depression and vapor pressure for 11 different polymers and surfactants (PEG 3350, 4000, 6000, 8000, 20000, PVP K12, K17 and K30, poloxamer 188, 388 and 407, HPMC E5, Na-CMC, polysorbate 20 and 80, vitamin E-TPGS, phospholipid, DOSS and SDS) in different concentrations.

### **Results**

Independently of the measuring method, an increase in osmolality with increasing concentration was observed for all polymers and surfactants, as would be expected due to the physicochemical origin of the osmolality. No correlation was found between the molecular weight of the polymers and the measured osmolality. The osmolality values were different for PVPs, PEGs, and Na-CMC using the two different measurement methods. The values obtained by the freezing point depression method tended to be similar or higher than the ones provided by vapor pressure, overall showing a significant difference in the osmolality measured by the two investigated methods.

### **Conclusions**

For lower osmolality values (e.g. surfactants), the choice of the measuring method was not critical, both the freezing point depression and vapor pressure could be used. However, when the formulations contained higher concentrations of excipients and/or

50 thermosensitive excipients, the data suggests that the vapor pressure method would be  
51 more suited.

52

53 **Keywords;** Osmolality, freezing point depression, vapor pressure, suspension,  
54 parenteral vehicles

## Introduction

Long-acting injectables (LAIs) are a unique drug formulation option that provide a slow, sustained release of the active pharmaceutical ingredient (API) after administration [1]. LAI formulations present several advantages over traditional oral formulations, including correct drug usage, reduced frequency of administration, enhanced therapy adherence and patient compliance as well as mitigation of possible adverse effects by avoiding peak plasma concentrations. Considering these properties, LAIs offer perspectives of improved quality of life for patients using these [2-7]. LAIs have attracted special interest in therapeutic areas such as schizophrenia, hormone replacement therapies, immunodeficiency virus (HIV), and tuberculosis, where repeated drug administration is required [6,8-11].

There are four main formulation classes of LAIs: i) oil solutions, ii) aqueous suspensions, iii) polymer-based microspheres/implants (including biodegradable and non-biodegradable), and iv) *in situ* forming gels/implants [12]. For the aqueous suspensions, stabilizers, polymers and/or surfactants, are added to the formulation to control the relative kinetics of particle growth of the system [13]. Their addition enables particle size reduction during milling, prevents particle agglomeration, and particle growth via Ostwald ripening of particles. This consequently impacts the overall stability, primarily physical, but also partly chemical, thereby supporting maintenance of the drug release profile over the shelf-life period [14, 15]. The stabilization mechanism can either be electrostatic repulsion or steric stabilization, hence the excipients used span a wide range of biocompatible charged and nonionic surfactants as well as polymers [15]. The selection of stabilizers is specific and crucial for each individual API [16]. Polysorbate 80, polysorbate 20, sodium dodecyl sulfate (SDS), and poloxamer 188 are examples of

surfactants used as stabilizers in aqueous suspensions[13]. Examples of used polymeric stabilizers include polyvinylpyrrolidone (PVP), sodium carboxymethylcellulose (NaCMC), poloxamer 338, and polyethylene glycol (PEG) 4000 [13, 17]. Some of these excipients are currently used in commercialized parenteral LAI formulated as aqueous suspensions, such as Invega Trinza, Aristada, Abilify Maintena, Depo-subQ Provera, see **Table I.**

For formulation of parenteral suspensions, a series of parameters should be considered to ensure formulation stability and safe administration with as little discomfort as possible for the patient. Such parameters include viscosity, pH, density, osmolality, and syringeability. Osmolality, as well as pH, is directly related to local irritation, pain, and/or endothelial damage. Osmolality is an estimation of the osmolar concentration of plasma, which is proportional to the number of particles per kilogram of solvent and is expressed as mOsmol/kg [23]. On the other hand, osmolarity (osmotic concentration) is defined as an estimation of the osmolar concentration of the plasma. This property is proportional to the number of particles per liter of solvent and its unit is mOsmol/L [24]. As only osmolality can be measured, a relationship between these two quantities has been determined through fundamental physical/chemical definitions. These definitions include the osmotic coefficient, i.e. a conversion factor particular to the solute system and the partial molal volume(s) of the solute(s) [24].

According to the United States Pharmacopeia (USP), the osmolality of blood ranges between 285 and 310 mOsmol/kg [25]. Generally, parenteral formulations should be isotonic (around 290 mOsm/kg) or moderately hypertonic (up to 500 mOsm/kg) [26], since hyperosmolality leads to a loss of water from the cells which causes cell shrinkage

and an increase in cellular viscosity, which will be associated with pain upon injection [23].

Osmometry to measure osmolality is, hence, an essential tool when characterizing the physicochemical properties of solutions for parenteral application [27]. The most common osmometers are based on the assessment of three properties of the solution: freezing point depression, vapor pressure, and osmotic pressure. The freezing point depression and the vapor pressure are the most commonly applied methods in commercial available equipment that measures osmolality [28]. Both methods provide a direct measurement of the osmolality and require a limited amount of sample. Furthermore, both methods are commonly used in pharmaceutical development as a fast, easy, and accepted method to determine the osmolality of parenteral formulations [29].

Some concerns have been raised regarding both osmometry techniques. Winzor [30] highlighted a disagreement observed for the osmolality measurements of PEG solutions by vapor pressure and freezing point depression [30], which was suggested to be due to the water adsorption by the filter paper disc, inherent to the vapor pressure technique [31]. However, this hypothesis did not consider the temperature effect on the excipients, specifically those that undergo temperature-dependent changes in hydration, such as surfactants and polymers used for stabilization of LAI suspensions. One example of these temperature-dependent polymers is PEG [32, 33]. While some osmolality data for solutions containing PEGs can be found in the literature [28, 34], there is a general lack of information about other excipients used to stabilize suspensions. Most of the publications published do not approach the subject of osmolality and tend to focus on the effects of different molecular weights of polymers, e.g. as stabilizers of amorphous solid

dispersions or in supersaturated drug solutions [35]. Lestari et al. [36] conducted a systematic screening of different surface modifiers for the production of physically stable nanosuspensions using wet ball milling. The group concluded that combinations of anionic surfactant and nonionic surfactant as well as combinations of anionic surfactant and polymeric stabilizer tend to be more successful for the formation of stable nanosuspensions. Furthermore, the study stated that the concentration and the principle of stabilization of surface modifier determines the formation of stable nanosuspensions [36], but in general no considerations were put on the osmolality. The purpose of the present study was therefore to study the osmolality of polymers and surfactants relevant for aqueous suspension-based LAIs at room temperature by two methods, i.e. the freezing point depression and the vapor pressure. The main goal was to provide general insights into the potential difference between the two methods across a broader range of excipients, particularly important for formulation purposes, as discussed above.

## **Theoretical approach to osmolality - considerations**

Osmolality provides an estimation of the concentration of solutes in a solution, and it can be assessed through any of the four colligative properties of the solvent [37]. When a solute dissolve in a pure solvent, specific changes, that are proportional to the solutes activity/concentration, occur in the solution's colligative properties [38], such as:

- the freezing point depresses
- the boiling point raises
- the osmotic pressure increases
- vapor pressure lowers [34]

In the section below a more detailed description of the freezing point depression and the vapor pressure are provided.



## Freezing point depression

Freezing point is defined as the temperature at which a solvent/solution will turn from liquid to solid. When the sample is added to the osmometer, it initially cools according to Newton's Law of cooling (the rate of cooling is proportional to the difference in temperature between the sample and its environment). However, for a mixture of solvent and solute (solution), the solution does not freeze, only the solvent.

When the freezing point is reached, the sample would remain at a constant temperature until all mass has been converted to the solid phase. However, solutions tend to supercool, meaning that the samples may cool below the freezing point temperature of the solvent until crystallization starts. As more solid is formed, the concentration of the solution increases at an exponential rate until it reaches a solubility limit – the eutectic point. Finally, all solvent becomes solid and the mass cools down to the equilibrium temperature (temperature plateau). During this period, the center of the sample alternates between thawing and freezing until it completely freezes and the sample slowly turns solid and cools to the equilibrium temperature [39].

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

$$\Delta T = K_f \cdot m \quad \text{Eq. 1}$$

where  $\Delta T$  corresponds to the temperature change from the pure solvent's freezing point to the freezing point of the solution,  $K_f$  is the freezing point constant (for water this is

1.86 °C/mol), and  $m$  is the molality of the nonvolatile solute, i.e. the osmolality of the solution at a particular molality of the solute in the particular solvent [38].

When measuring the freezing point depression, the sample is vibrated intensely for a moment, after a fast supercooling of the solvent to a predetermined temperature, which produces heat of fusion as crystallization occurs. When a plateau of the cooling curve is reached, its value is measured by a thermistor. This plateau tends to be below the freezing point of the pure solvent, as explained above, but by relating the unknown with standard solutions, the osmolality can be determined [38-40].

This measurement does not provide any information regarding the nature of the particles (e.g. size, shape or conformation), as the calculation only depends on the number of particles in solution. However, according to Sweeney and Beuchat [41], Eq. 1 is supported by a series of assumptions that are often violated, since the relationship between the freezing point depression and osmolality differs between solutes and solvents [42]. The freezing point depression constant not only varies between solvents, but also within the same solvent as a function of solute, i.e. the type and concentration of solute [42]. Furthermore, the value provided by this technique can deviate from the real value for three different reasons:

1. violation of thermodynamic assumptions (i.e. solution is very dilute and presents ideal behavior)
2. temperature dependence of the solute solubility
3. mathematical simplifications for osmolality calculations

## **Vapor pressure**

In osmolality measurements made using the vapor pressure method, the sample is inoculated onto a solute-free paper disc in the sample holder, the sample holder is pushed inside the instrument and the chamber is closed [38]. The sensing element is a thermocouple hygrometer composed of two thermistors with a sample holder in between. When the sample is added to the sample holder, it is placed in between these two thermistors [38]. As vapor pressure equilibrates in the chamber airspace, the thermocouple senses the ambient air temperature, which will be the reference point for the measurement. Afterwards, the thermocouple is cooled until a temperature below the dew point. As a consequence, the solvent condenses in the chamber and forms small droplets on the surface of the thermocouple. At this point, the temperature of the thermocouple is controlled by the water condensing onto its surface. As water continues to condense, the thermocouple temperature tends to increase until the dew point is reached. At the dew point, water condensation stops and, consequently, the thermocouple temperature stabilizes giving an output proportional to the differential temperature (dew point temperature depression) – which is a function of the solution vapor pressure. In this context, the chemical potential of the solution's solvent can be compared with the one of the solvents alone [43].

The relationship between sample osmolality and the reading obtained by the osmometer is governed by fundamental considerations. Vapor pressure depression is a linear function of osmolality, since it is one of the colligative properties of a solution. The relationship between vapor pressure depression and the dew point temperature is given by Equation 2 [43].

$$\Delta T = \frac{\Delta e}{S} \quad \text{Eq. 2}$$

where the osmotic pressure,  $\Delta T$ , is the dew point temperature depression in degrees Celsius,  $\Delta e$  is the difference between saturation and chamber vapor pressure, and  $S$  corresponds to the slope of the vapor pressure temperature function at ambient temperature [43].

$S$  is determined by the Clausius-Clapeyron equation (Equation 3), as a function of temperature ( $T$ ), saturation vapor pressure ( $e_0$ ) and latent heat of vaporization ( $\lambda$ ) [43].

$$S = \frac{e_0 \lambda}{RT^2} \quad \text{Eq. 3}$$

where  $R$  is the universal gas constant. The dew point temperature depression,  $\Delta T$ , is measured as a voltage signal from the thermocouple and the signal is processed to display the reading. This voltage is equal to  $\Delta T$  multiplied by the thermocouple responsivity, which is approximately 62 microvolts per degree Celsius. After voltage amplification by a preamplifier, the microprocessor processes the voltage signal to provide calibration and compensation functions and then displays the reading in mmol/kg [43].

When comparing the freezing point depression and the vapor pressure method, one clear advantage of the latter is that it does not involve a change in the physical state of the solution. Additionally, this technique can be performed in a wide temperature range, and it is not affected by temperature-sensitive changes in solute solubility. Furthermore, viscosity and/or presence of suspended particles does not influence the measurement. Nonetheless, it is important to mention that the vapor pressure technique is less suitable when volatile or organic solvents are present in the solution, as it will influence the equilibrium reached. Overall, theoretically, the vapor pressure method seems to have a

much broader range of minimal error applications when compared to freezing point depression [38].

## **Materials and methods**

### **Materials**

Hydroxypropylmethylcellulose 2910, 5 mPas (HPMC E5) was purchased from DDP Specialty Electronic Materials (DDP Specialty Electronic Materials Plaquemine, LA, USA). Poloxamer 188, poloxamer 338 parenteral, poloxamer 407, Polyvinylpyrrolidone (PVP) K12 parenteral, PVP K17 parenteral, PVP K30, and sodium dodecyl sulfate (SDS) were acquired from BASF (BASF Chemtrade MBH, Germany). Polyethylene glycol (PEG) 4000 parenteral was sourced from Clariant (Clariant International Ltd, Switzerland). PEG 3350 was bought from Spectrum (Spectrum Chemical MFG Corp, CA, USA), PEG 6000 Flake was purchased from Dow Chemical (The Dow Chemical Company, MI, USA), and PEG 8000 was sourced from Sigma-Aldrich (Sigma Aldrich corporation, MO, USA). PEG 20000 was bought from Merck (Merck KGaA, Germany). D- $\alpha$ -tocopheryl polyethylene glycol 1000 succinate (Vitamin E-TPGS) was acquired from Isochem (France). Polysorbate 20 parenteral and polysorbate 80 were purchased from Croda Inc. (NL, USA). Docusate sodium was bought from Cytec (Cytec Industries, Netherlands). Sodium carboxymethylcellulose (Na-CMC) was acquired from Ashland Inc. (France). Lipoid E PG was sourced from Lipoid GmbH (Lipoid GmbH, Germany). Purified water was freshly prepared using a Milli-Q®integral water purification system (Milli-Q Advantage A10; MerckMillipore, Merck A/S, Denmark).

The chemical structures and key physicochemical information regarding the polymers and surfactants used in this study can be found in **Supplemental information - Tables S1 and S2**, respectively.

## **Methods**

### **Preparation of excipient solutions**

All solutions were initially prepared in glass beakers with approximately 80% of the total volume (100 mL) (**Table II**). The samples were magnetically stirred overnight (300 rpm, 21 °C), protected from light by wrapping beakers in aluminum foil. The volumes were adjusted to 100 mL with deionized (DI) water on the next day after the complete dissolution of the excipient.

### **Osmolality by Freezing Point Depression**

The osmolality was measured using OsmoPRO (Advanced® Instruments 3250, Norwood, MA, USA), an osmometer based on the freezing point depression principle. The accuracy of the osmometer was confirmed at the start and completion of each testing session by assaying a reference solution of known osmolality provided by the manufacturer, 290 mOsm/kg (Advanced® Instruments 3250, Norwood, MA, USA). All samples were equilibrated to standard laboratory temperatures (20-21 °C) before assessment. Osmometry was performed in triplicate using a 20 µL sample.

### **Osmolality by Vapor Pressure**

The osmolality measurements based on vapor pressure were performed using VAPRO (Wescor, Inc 370 West, Utah, USA). The osmometer was calibrated with three concentrations of standard salt solutions (100, 290, and 1000 mOsm/kg) before starting

the measurements. Also, for all measurements, solutions were equilibrated to standard laboratory temperatures (20-21 °C) before assessment. Osmometry was performed in triplicate using a 10 µL sample.

## **Statistical analysis**

All tests were conducted at least in triplicate. The results are reported as the average value with standard deviation for each solution. Statistical analysis was performed using a Bayesian bivariate mixed model, since it was verified that the concentration effect on the osmolality values differences could not be seen as a random variation. The Bayesian bivariate mixed model is a multivariate linear mixed effect model, which combines the strength of the paired t-test, by matching the measurements on samples, and on the other hand, similar to classical ANOVA. This approach allowed us to model the paired differences by accounting for the concentration effects. A detailed description of the model can be found in the **Supplemental Information**.

## **Results and discussion**

Parenteral LAIs can be injected via different administration routes depending on a combination of anatomical, physiological, and physical factors. Most commercially available LAI are injected intramuscular (IM), but other administrations routes may also be relevant, e.g. subcutaneous (SC) and intravitreal (as shown in **Table I**). The administration route is chosen according to the intended therapeutic action [44,45].

Adjustment of the osmolality of parenteral products is critical for patient comfort and safety when these drug products are administered. In this work, the focus is on surfactants and polymers used to stabilize suspensions.

## **Osmolality of different aqueous solutions of polymers used as stabilizers in suspensions**

The polymers investigated in this study were PEG 3350, 4000, 6000, 8000, and 20000; PVP K12, K17, and K30; poloxamer 188, 338, 407; HPMC E5, and Na-CMC. In general, PEG and Na-CMC are known suspending agents, while PVP, poloxamers and HPMC are classified as wetting agents. Therefore, the functionalities of the investigated excipients differ. However, no matter their function in the formulation, it is important to understand their contribution to the final obtained osmolality in the solution/suspension they may be a part of, in order to design the best formulation composition.

The data obtained for the osmolality of the investigated polymers, measured by both the freezing point depression and the vapor pressure method, are presented in **Fig. 1**. Independently of the measuring method, an increase in osmolality with increasing concentration was observed for all polymers, as would be expected due to the physicochemical origin of the osmolality (see **Section 2**). Furthermore, no increase in osmolality with molecular weight for the same excipient concentration was observed. For example, by taking the 10.5% w/v concentration for PEGs, the average osmolality values were 124.0, 114.3, 110.0, 85.0 and 99.3 for PEG 3350, PEG 4000, PEG 6000, PEG 8000 and PEG 20000, respectively (see **Fig. 1** and **Supplemental information - Table S3**). This finding contradicts the trend presented in already published data on PEGs [28, 30, 31]. When the osmolality measured was plotted as a function of the four concentrations,



an exponential increase in osmolality was observed with increasing concentration for all polymers (see **Fig. 1**). This non-linearity for osmolality may be a reflection of the limited connection between the chemical potential in the solutions and the molar concentration, but also other important factors such as size, shape, and hydrophobicity of the polymer chain may explain the observed discrepancy. Another possible explanation can be the change from dilute to semi-dilute regimes. For polymers in a dilute regime, it is considered that each polymer coil/particle is independent and not in contact with each other. However, when the same polymer enters its semi-dilute regime, individual coils may be in contact with each other, i.e. it is not anymore possible to detect individual coil particles. Furthermore, when the solutions enters into a concentrated regime, coils entangle with each other, leading to the destruction of individual particles [46-48]. Having said this, from the collected data in this work it may be suggested the PVP was always in a dilute regime (i.e. individual particles) in the concentration range studied, while PEG was in a semi-dilute regime (i.e. entangled polymer chain, no individual particles), leading to different behavior as result of concentration between these polymers.

By comparing the osmolality values of the different polymers, it was observed that the PVPs, i.e. PVP-K17, and PVP-K30, had osmolality values similar to those of Na-CMC, although Na-CMC has a much higher molecular weight than PVPs (see **Table S1**). On the other hand, it was noticed that the different PEGs had osmolality values comparable to the investigated poloxamers and HPMC E5. The comparable values observed for the poloxamers were probably due to the similarity in composition of these polymers with PEG, though the molecular weight of the poloxamers tends to be lower. HPMC E5 has a higher molecular weight (i.e. 20000 g/mol) when compared to the other two polymers, PEGs and poloxamers (between 3000 and 18000 g/mol) and presents osmolality values similar to the PEGs with higher molecular weight (i.e. PEG 20000). Additionally,

comparison between Na-CMC and HPMC was performed, since both are cellulose-based polymers, that differ in charge, anionic versus non-ionic nature, respectively, which may be a possible and more probable explanation for the difference in osmolality observed (e.g. at 4 %w/v: 10-20 mOsm/kg for HPMC vs. 95-100 mOsm/kg for Na-CMC). At this stage, it is important to state and draw attention to the fact that the charge of all substances, whether anionic, cationic or nonionic, will have some effect on the surface tension when added to the aqueous solvent. This could contribute to the physicochemical properties of the system under investigation and, consequently, affect the measured value for osmolality.

The data collected by the vapor pressure technique showed similar trends to the freezing point depression technique with respect to the molar dependency between the polymers, though with some differences, as can be seen by analyzing the different graphs presented in **Fig. 1**. The osmolality values were different for PVPs, PEGs, and Na-CMC using the different measurement methods. It could be seen that the values given by the freezing point depression method tended to be similar or higher than the ones provided by vapor pressure. However, the differences observed were statistically significant, so before defining the technique intended to be used for the osmolality analysis, a careful analysis should be performed with respect to which method that would be most suitable for determining the osmolality of LAIs suspensions that contain those specific polymers. For suspensions it is common use to add more than one excipient, but since the surfactant contribution to the osmolality tends to be relatively low as is discussed below, the main contributors towards osmolality would be a potentially added polymer. In a suspension, the API would also have a limited contribution to the osmolality.

Na-CMC was present in a much lower concentration compared to the other polymers investigated. The osmolality values obtained for the poloxamers were different from the osmolalities measured for HPMC E5 (see **Fig. 1** and **Supplemental information - Table S3, Fig. S1**). For example, for HPMC solutions osmolality was zero up to 10 % w/v, while some poloxamers show osmolality of about 20-30 mOsm/kg at 7 % w/v (see **Fig. 1** – HPMC compared to poloxamer 188 or 338) [49,50].

Based upon the reports from literature there was a reason to assume that there would be a correlation between the molecular weight of PEG and osmolality value, which was not observed in the present study nor for any of the other investigated polymers, i.e. PVP and poloxamer. As can be seen in **Fig. 2**, there was no clear correlation between molecular weight of PEG and osmolality value, and the osmolality values in between PEGs did not vary much at the same concentration, in the present study.

Overall, when comparing the osmolality values obtained by both techniques, it was clear that the values determined by vapor pressure were in general lower than those determined by freezing point depression. The difference in the values observed for both techniques was most pronounced for PEG 20000, PVPs, poloxamers, and HPMC E5. Possible explanations for the differences might be due to micelle formation for poloxamers. Thermoresponsive properties or viscosity changes influence the osmolality measurement as discussed above, which can also be influenced by the molecular weight of the polymer and the concentration of excipient in solution [30]. According to Ashland's product properties sheets the dynamic viscosity of a 1 % w/v solution in water of PVP-K12 and PVP-K30 is 10-14 mPa.s and 27-33 mPa.s, respectively [51]. As shown in **Fig. 1** and **Supplemental information - Table S3**, the osmolality measured by the freezing point depression and the vapor pressure methods was, at the concentration of 3.5 % w/v, 29.3

425  $\pm 0.6$  mOsm/kg and  $20.7 \pm 2.3$  mOsm/kg for PVP K12, respectively; and  $14.3 \pm 1.2$   
426 mOsm/kg and  $3.7 \pm 1.2$  mOsm/kg for PVP K30, respectively, which does not support  
427 viscosity as an important factor for the difference observed. Sweeney and Beuchat [41]  
428 have discussed the same hypothesis from a theoretical perspective and claimed that the  
429 sample dynamic viscosity differences in principle violates the thermodynamical  
430 assumptions of osmolality determination. This means that for the freezing point  
431 depression method, the cryoscopy constant ( $K_f$ ) may deviate from the 1.86 K/(mol/kg)  
432 often used (see **Section 2**) [41]. Additionally, Michel and Kauffmann [52] demonstrated  
433 a temperature dependency of the osmolality of PEG 6000, supporting the theoretical  
434 analysis made by Sweeney and Beuchat [41], and in accordance with the differences  
435 observed between the freezing point depression and the vapor pressure methods in the  
436 present work. These inconsistencies might be extrapolated to other molecules with  
437 temperature-dependent behaviors, such as poloxamers and HPMC.

438  
439 The difference in osmolality found between the two measurement methods tended to be  
440 approximately constant across all the PEGs, with a slight increase across the different  
441 molecular weights, with increasing polymer concentration (see **Supplemental**  
442 **information - Table S3**). Comparable observations were seen for the PVPs, Poloxamers,  
443 and HPMC E5, whereas less difference was seen for Na-CMC. It can be concluded that  
444 molecular weight plays a critical role when it comes to osmolality determination for most  
445 of the polymers. However, the information provided by the supplier on the molecular  
446 weight are the average of the polymer composition which then could generate deviances  
447 in the value osmolality between suppliers.

## Osmolality of different solutions of surfactants used as stabilizers in suspensions

The osmolality values for solutions of surfactants measured by both the freezing point depression and the vapor pressure method are presented in **Fig. 3**. As observed for the polymers, the osmolality for the surfactant solutions also increased with increasing excipient concentrations. For the freezing point depression technique, a higher osmolality was measured for polysorbate 20 than for polysorbate 80, which can be due to the saturated chains in polysorbate 20 relative to the unsaturated double bond in polysorbate 80 (see **Table S2**), producing a lower chemical activity [53]. Additionally, polysorbates present similar values of osmolality for similar concentrations of PEG (i.e. 4% w/v polysorbate vs 3.5% w/v PEG), which might be explained by the fact that polysorbates contain PEG as a part of their molecular structure (**Support Information – Table S1-S2**). Lipoid E PG and Vitamin E-TPGS are surfactants with none to marginal contributions to the osmolality of a solution, but also the contribution of the two investigated polysorbates was very limited. In parenteral formulations antioxidants (e.g. ascorbic acid, citric acid), preservatives (e.g. benzoic acid, phenol), and potentially chelating agents (e.g. disodium edetate, detate calcium disodium) may be used. As presented in the work published by Rayaprolu et al. [54] the concentrations used of these agents, 0.001-2% w/v, is so low that their impact on the osmolality is not considered significant [54,55].

DOSS and SDS contributed to the osmolality, as shown in **Fig. 3** and osmolality values are also presented in the supplemental information (**Table S4**). While the other surfactants are non-ionic, DOSS and SDS are anionic surfactants, and ionize when in water which may explain why these two surfactants behaved differently than the others investigated.

It is important to refer that although this observation was clear for SDS and DOSS, almost all substances ionize to some extent, which can partly also influence the determined osmolality values. The osmolality values recorded for the surfactants by the vapor pressure method were lower than the measurements obtained by the freezing point depression method for all investigated surfactants, as was observed with the polymers. Nonionic surfactants are in general known to have temperature dependent CMC values [56], i.e. this may at least partly explain the difference between the two methods (see **Fig. 3** and **Supplemental information - Table S4**) [56]. The observations were in accordance with data published by Kiyosawa [28] and Windsor [30] with respect to PEG. Both authors suggested that the observed discrepancies were caused by the different temperatures applied in the two measurement methods, i.e. referring to the temperature dependency of the measurement. As explained above, osmolality is a measurement of the number of solute molecules dissolved in solution. The vapor pressure method is based on the temperature difference recorded to achieve a stable vapor pressure inside the chamber, hence the method may be less reliable at the lower concentration range, i.e. lower osmolality range (see **Section 2**). However, when it comes to defining an osmotic parenteral formulation, this would be less critical, as the concentrations of the excipients used in the formulations tend to be higher and osmotic agents may be added. These osmotic agents can be water-soluble salts of inorganic acids (e.g. magnesium chloride or sulfate; sodium, or potassium), water-soluble salts of organic acids (e.g. sodium and potassium acetate, sodium benzoate, sodium citrate, sodium ascorbate), carbohydrates (e.g. xylose, glucose, mannose, sucrose, maltose), water-soluble aminoacids (e.g. glycine, leucine, alanine, methionine, etc.) or organic polymers (e.g. hydroxyethyl, methylcellulose, cross-linked PVP, polyethylene oxide, polyacrylamides, etc.) [54].

## Comparison of osmolality values of excipients

The data presented in **Figs. 1** and **3** (see also **Supplemental information - Table S3-S4**) demonstrated that the osmolality values obtained for the surfactants were generally lower than those obtained for polymers, except for SDS which gave osmolality values comparable to the lower polymer concentrations. Also, the different polysorbates, poloxamers and PEGs had almost the same osmolality at the same concentration. The difference observed in the osmotic contribution was most likely a reflection of the different interaction with the aqueous phase for the two classes of excipients, i.e. hydration of molecules (polymers, some surfactants) versus hydrophobic surfactants. Furthermore, ionic versus non-ionic substances, the small ions (e.g.  $\text{Na}^+$ ) tend to have a significant impact on increasing osmolality, as shown by comparing osmolality values to HPMC and Na-CMC in this work (see **Fig. 1**). The same was applicable to SDS and DOSS, which showed the highest osmolality, for a given concentration, among the surfactants tested.

As discussed above (see **Supplemental information - Table S4**) for the lower concentrations of polysorbate 20, polysorbate 80, and all concentrations of Vitamine E-TPGS and Lipoid E PG, the differences between the osmolality values estimated by the two techniques can be overlooked, simply because the values were almost zero for the majority of the concentrations of surfactants studied. For the higher concentrations of polysorbate 20 and 80 as well as DOSS and SDS some tendencies towards a difference were observed (see **Supplemental information – Fig. S2**).

Furthermore, a greater difference was observed between the two measurement methods for the polymers. To better understand the difference between the two osmometry

methods a statistical analysis was made for the following groups of polymers investigated in this study, namely PEG (3500, 4000, 6000, 8000, 20000), poloxamer (188, 338, 407), and PVP (K-12, K-17, K-30). The results are presented in **Fig. 4**.

As it can be seen in **Fig. 4**, the difference in osmolality measured by freezing point depression and vapor pressure for these three groups of polymers was significant for PEGs and poloxamers, since the confidence intervals for the two different osmometry methods do not overlap. The difference was greatest for PEG, followed by poloxamer, i.e. the findings for PEG reported here and in the literature [30-32] may also be extrapolated to other polymer classes.

When evaluating the data presented in this study, it was clear that for lower osmolality values that the freezing point depression method seemed most accurate for systems without thermosensitive excipients. However, as the osmolality became higher and reached the relevant range for injectable formulations, large differences were observed depending on the method applied. When working with formulations with thermosensitive excipients it therefore would be recommended to use the vapor pressure method. For very dilute formulations, the method used may be less critical, however, it is in general recommended to consider carefully which method to use.

## **Conclusion**

The present study showed a dataset of osmolality values for a range of excipients, e.g. including polymers and surfactants that may be used in parenteral formulations. When designing a formulation comprising of drug nano/microsuspensions, the contribution of



the excipients on the osmolality should be taken into account with respect to obtaining an isotonic drug product or the targeted tonicity.

The non-linearity of osmolality and difference between the two osmometry methods investigated was most probably a reflection of the limited relationship between the chemical potential in the solutions and the activity of the polymers, micellar formation for surfactants, thermoresponsive properties influencing the measurement, viscosity of the solution, molecular weight of the excipients, the ionic charge of the excipients and the concentration of excipient in solution.

The osmolality values obtained for the surfactants were generally lower than those obtained for polymers, which reflects the different interactions with the aqueous phase for the two classes of excipients, e.g. micelle formation versus solubilization.

The data presented in the present study shows that for lower osmolality values, the freezing point depression method seemed more accurate for systems without thermosensitive excipients than the vapor pressure method. However, as the osmolality became higher and in the relevant range for injectable formulations large differences between the two methods were observed. When working with formulations containing thermosensitive excipients it is recommended to use the vapor pressure method.

## **Supplementary information**

The online version contains supplementary material.

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## **Conflict of Interest statement**

The Authors declare that they have no conflict of interests to disclose.

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## Figures Legends

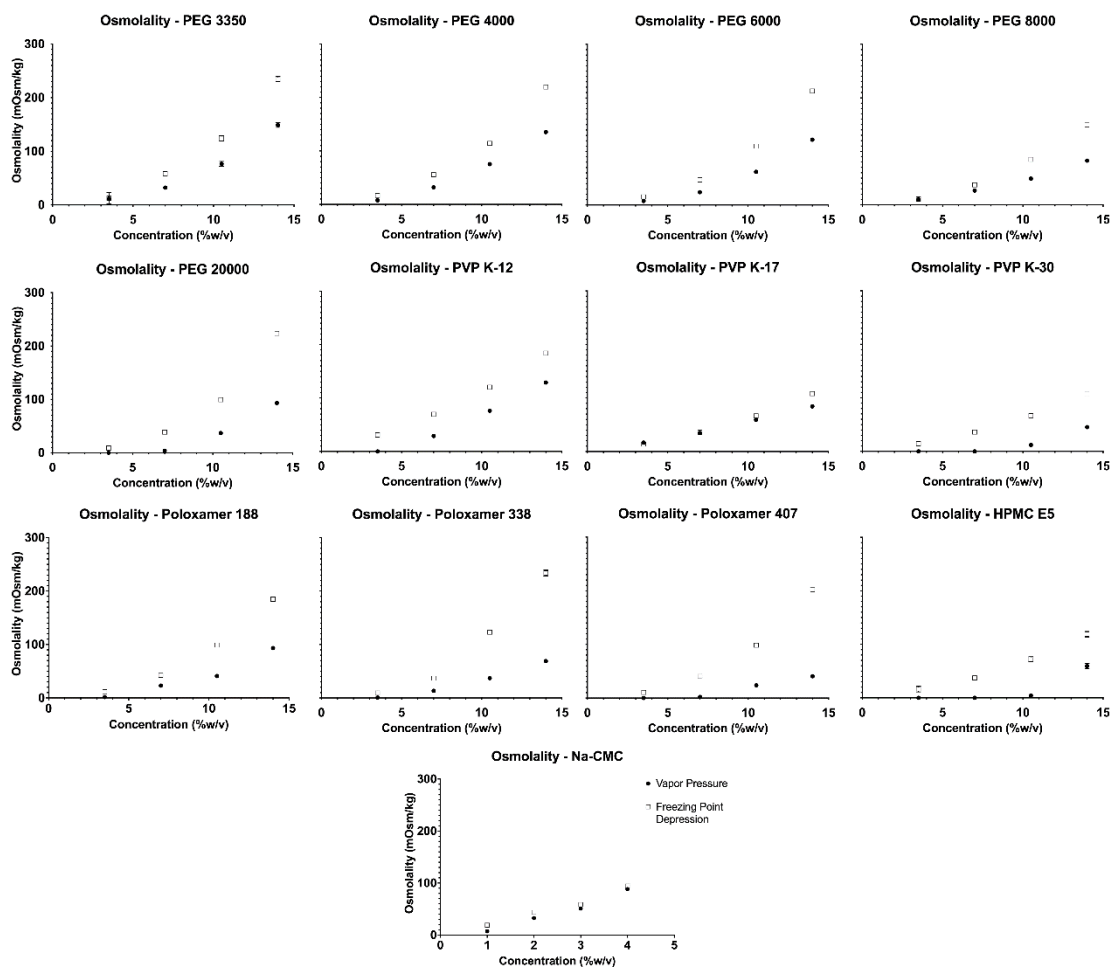
**Fig. 1** Overview of the osmolality values of polymer solutions prepared with respective concentrations in percentage weight per volume (% w/v) and the difference between the two measuring principles based on the average values and respective standard deviations: freezing point depression (empty square) and vapor pressure (full circle).

**Fig. 2** Plots of osmolality by vapor pressure (left) and freezing point depression (right) against concentration for the different molecular weights of the same polymers on one graph – a) PEGs, b) PVPs, and c) poloxamers.

**Fig. 3** Overview of the osmolality values for surfactant solutions prepared with respective concentrations in percentage weight per volume (% w/v) and the difference between the two measuring principles based on the average values and respective standard deviations: freezing point depression (empty square) and vapor pressure (full circle).

**Fig. 4** Graphs show the estimated osmolality and respective 95% confidence interval for the vapor pressure or freezing point depression method grouped by concentration, for three different excipients: a) PEGs, b) PVPs, and c) poloxamers.

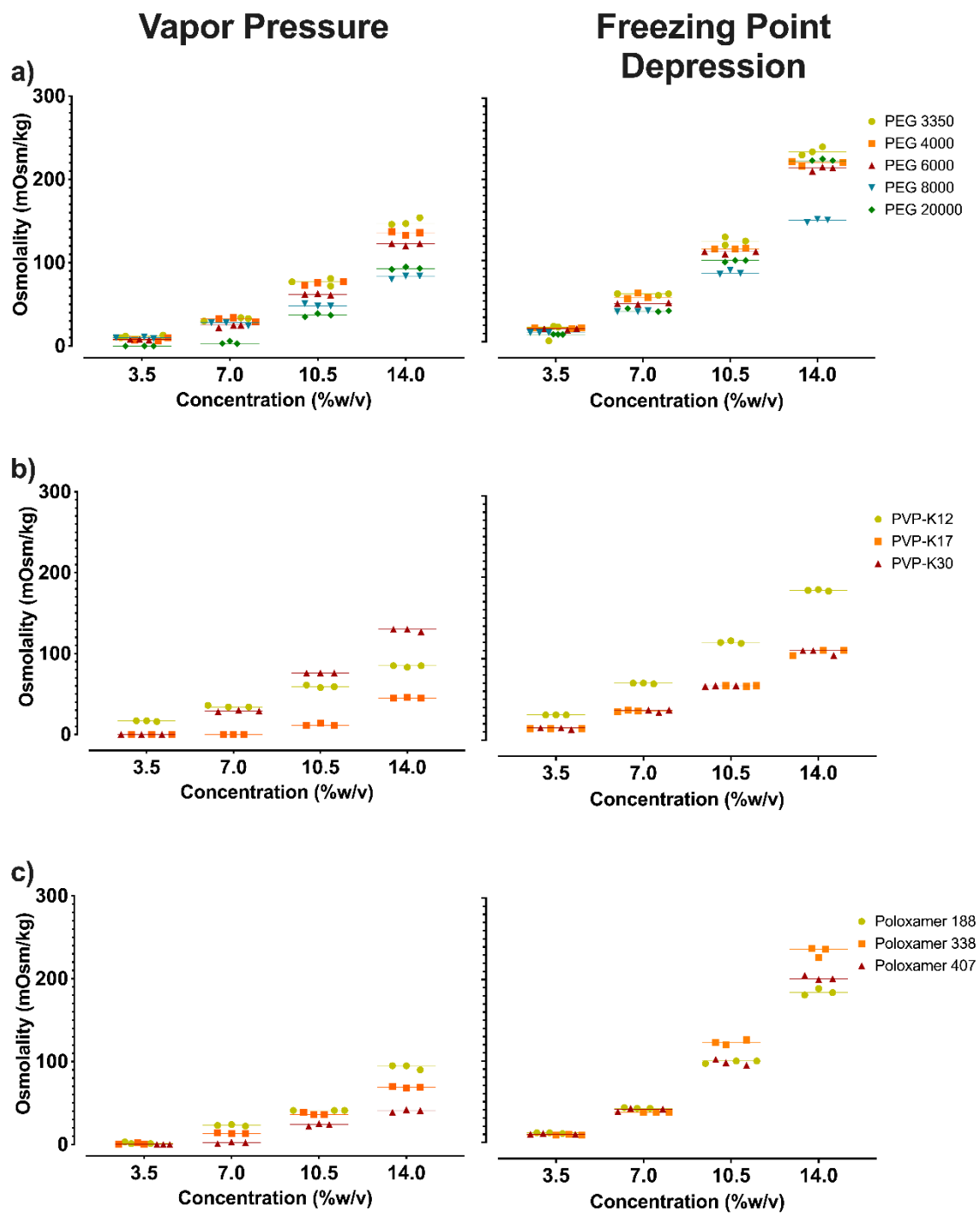
751 **Figure 1**



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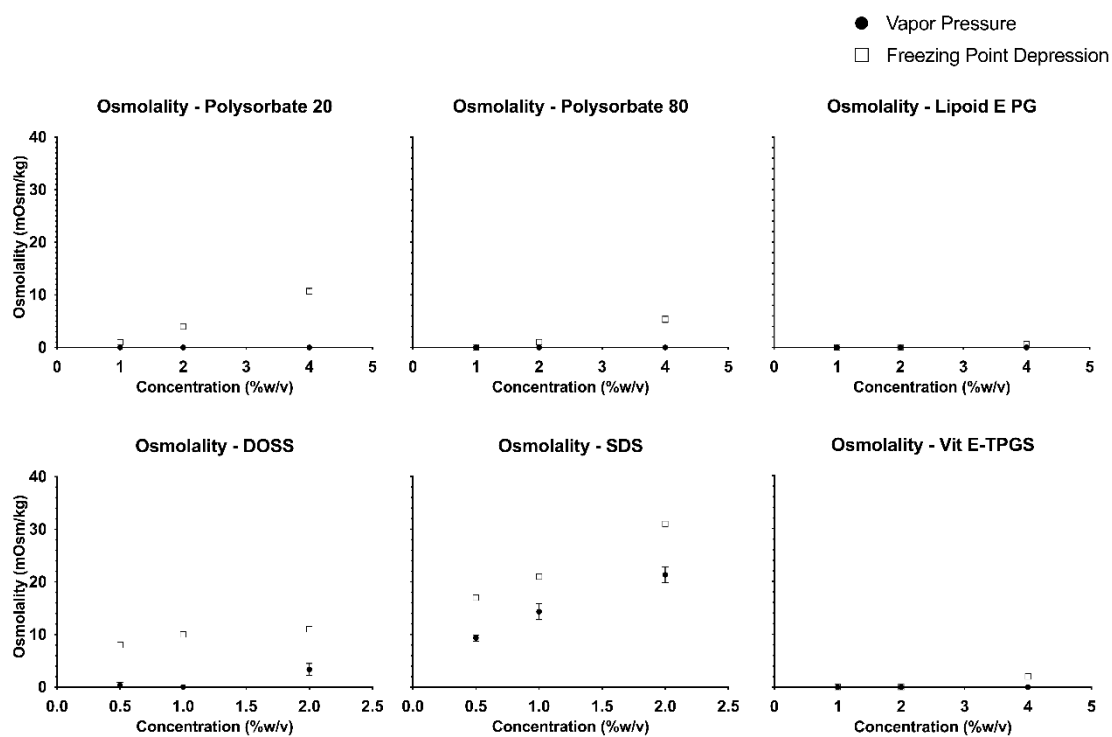
754 **Figure 2**



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757 **Figure 3**



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**Figure 4**

