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Letter

Reappraisal of disparities between osmolality estimates by freezing point depression and vapor pressure deficit methods

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Abstract

As a response to recent expression of concern about possible unreliability of vapor pressure deficit measurements (K. Kiyosawa, Biophys. Chem. 104 (2003) 171–188), the results of published studies on the temperature dependence of the osmotic pressure of aqueous polyethylene glycol solutions are shown to account for the observed discrepancies between osmolality estimates obtained by freezing point depression and vapor pressure deficit osmometry – the cause of the concern.

Keywords: Freezing point depression; Polyethylene glycol; Second virial coefficients; Vapor pressure deficit

1. Introduction

Attention has been drawn recently [1] to the disagreement between estimates of osmotic pressure for aqueous polyethylene glycol solutions from freezing point depression and vapor pressure deficit measurements. This corroboration of earlier evidence [2–4] was taken to imply the unreliability of one of the methods used for osmotic pressure determination. Indeed, an increase in polymer concentration as the result of water adsorption by the filter paper disc [5] was considered to be a potential source of error in vapor pressure deficit osmometry. However, such interpretation of the disparity between osmolality estimates by freezing point depression and vapor pressure deficit procedures disregards the consequences of any effect of temperature on the solute under investigation. This is an important omission for polyethylene glycol, which is known to undergo temperature-dependent changes in hydration and conformation [6,7]. The purpose of the present communication is to assemble published data [1,3,8,9] that identifies the latter explanation as the source of the discrepancies between the osmotic characteristics of polyethylene glycol deduced by the freezing point depression and vapor pressure deficit procedures.

2. Theoretical considerations

Thermodynamic equivalence of the two methods is emphasized by the ability to describe the osmot-

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ic pressure (π) in freezing point depression and vapor pressure deficit osmometry by the same basic expression, namely,

$$\frac{(\pi)_P}{RT_0} = \frac{\lambda(T_0 - T_A)}{T_0 T_A R M_s} = m_A(1 + C_{AA} m_A + \ldots) \qquad (1)$$

where thermodynamic non-ideality of a solution with molal concentration m_A is expressed in terms of the molal second virial coefficient for solute self-interaction C_{AA} [10]. Use of the molal concentration scale is dictated by the constraint of constant pressure that applies to the osmotic pressure measurement [10,11]. R is the universal gas constant and M_s the molar mass of solvent. For freezing point depression measurements, λ is the molar latent heat of fusion of solvent, and T_0, T_A the respective freezing points of pure solvent and a solution with concentration m_A of solute. In vapor pressure deficit osmometry, λ is the molar heat of vaporization of solvent, whereas T_A is the dewpoint of the solution with concentration m_A at the temperature of interest, T_0. The substitution of T_0^2 for $T_0 T_A$ in the denominator of Eq. (1) is a valid approximation that is usually made in both procedures.

Since the heat of vaporization is considerably greater than the latent heat of fusion, the difference between T_0 and T_A is much smaller in vapor pressure deficit osmometry. Specifically, $\lambda/(T_0^2 R M_s)$ is 0.303 for the vapor pressure deficit method at 25 °C; and 1.862 for freezing point depression studies ($T_0 = 0$ °C). The basic measurement in either form of osmometry is $(T_0 - T_A)$; but the quantity returned by current instruments is the parameter O_A obtained from the expressions

$$O_A = (T_0 - T_A)/0.303;$$

vapor pressure deficit ($T_0 = 298.15$ K) (2a)

$$O_A = (T_0 - T_A)/1.862;$$

freezing point depression ($T_0 = 273.15$ K) (2b)

where O_A (which has the dimensions mol/kg) is termed the osmolality of the solution with molal concentration m_A. From Eq. (1), it is evident that

$$O_A = (\pi)_P/(RT_0) = m_A(1 + C_{AA} m_A + \ldots) \qquad (3)$$

in which the osmotic pressure is expressed in MPa and $RT_0 = 2.479$ kg MPa mol^{-1} at 25 °C. Eq. (3) provides the logic for regarding the osmolality as the molality of a solution under conditions of thermodynamic ideality ($C_{AA} = 0$). However, the osmolality differs from the molal thermodynamic activity (a_A) of the solution, which is given by the relationship [10–12]

$$a_A = m_A(1 + 2C_{AA} m_A + \ldots) \qquad (4)$$

Nevertheless, determination of the osmolality provides a valid measure of thermodynamic non-ideality in that it renders feasible the assignment of a magnitude to C_{AA}.

Unlike its molar counterpart (B_{AA}), the molal second virial coefficient (C_{AA}) is not normally expressible on the statistical–mechanical basis of excluded volume. For incompressible solutions, however, C_{AA} is related to B_{AA}, the parameter deduced from osmotic pressure measurements made under the constraint of constant solvent chemical potential, by the expression

$$C_{AA} = (B_{AA} - M_A \bar{v}_A)/\rho_s \qquad (5)$$

where the product of molar mass (M_A) and partial specific volume ($\bar{v}_A$) of the solute takes into account the molar volume of the unsolvated solute. The solvent density (ρ_s) makes allowance for the different dimensions of C_{AA} (kg/mol) and B_{AA} (l/mol).

Whereas the above theory is expressed in terms of molal concentration and the corresponding second virial coefficient, the usual experimental practice in freezing point depression and vapor pressure deficit experiments entails measurement of O_A as a function of solute concentration defined on a g/kg basis–thereby avoiding the need to assign a magnitude to M_A for the calculation of m_A. Under those circumstances, the counterpart of Eq. (3) is most conveniently written as

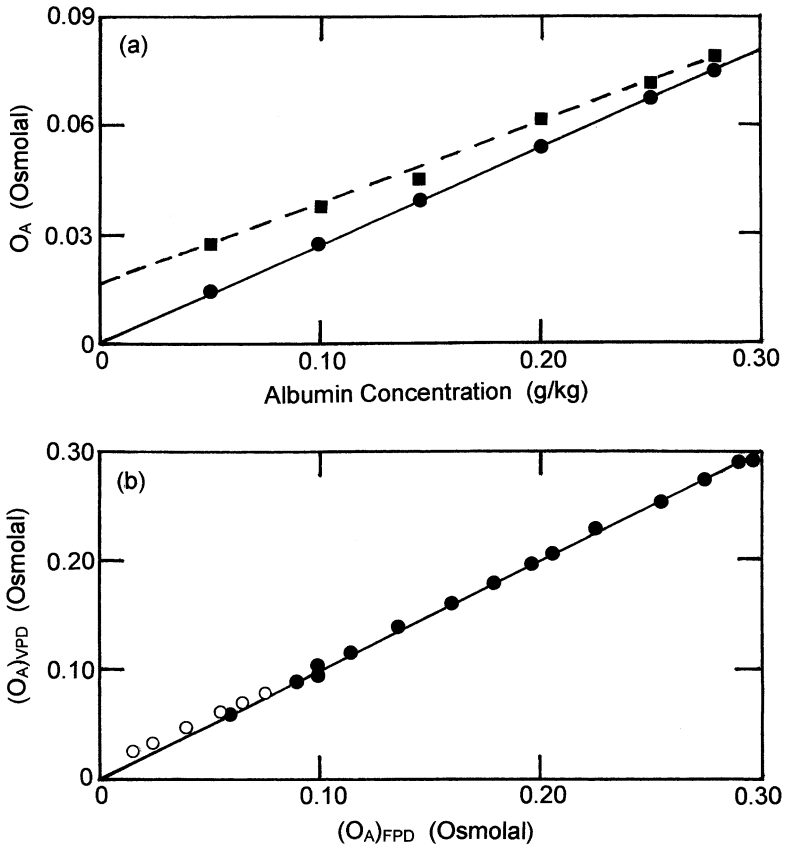

Fig. 1. Comparison of osmotic behavior deduced from freezing point depression (FPD) and vapor pressure deficit (VPD) measurements. (a) Dependence upon albumin concentration of the osmolalities determined by freezing point depression (●) and vapor pressure deficit (■) methods. (b) Calibration plot establishing equivalence of the procedures for determining the osmolality of salt solutions (●): open symbols denote the additional data from Fig. 1a, which essentially reflect the osmotic behavior of NaCl present as counterions in the albumin solutions. (Data taken from Ref. [1]).

$$\frac{O_A}{w_A} = \frac{(\pi)_P}{RT_0 w_A} = \frac{1}{M_A} + A_2 w_A \ldots \quad (6)$$

where $w_A = M_A m_A$ is the weight-concentration counterpart of the molal solute concentration; and where $A_2 = C_{AA}/M_A^2$.

3. Consideration of experimental results

As noted in Section 1, this investigation stems from concerns expressed recently [1] about inconsistencies between estimates of osmolality by freezing point depression and vapor pressure deficit methods. The thermodynamic equivalence of the two procedures precludes any theoretical reason for the return of an invalid measurement by either method. That possibility is also ruled out by the good agreement observed between the two osmolality estimates for salt (NaCl, KCl, $CaCl_2$) and sugar (glucose, sucrose, raffinose) solutions [1]. It is therefore necessary to consider more closely the two systems for which disparate osmotic behavior was reported [1].

3.1. Bovine serum albumin

The osmolalities of aqueous bovine serum albumin solutions obtained by freezing point depression (●) and vapor pressure deficit (■)

measurements [1] are presented in Fig. 1a, which certainly reveals an increasing discrepancy between estimates with decreasing protein concentration. In as much as the macromolecular contribution to O_A is only 4–5 milliosmolal at the highest protein concentration, the measured values are mainly reflecting the presence of salt (Na^+ and Cl^- counterions) in the albumin sample. It may therefore seem surprising to observe such disparities for solutions that differ in only minor respects from the salt solutions for which good agreement between osmolality estimates was observed [1].

Closer examination of Fig. 1a shows that the major divergence of vapor pressure deficit measurements from their freezing point depression counterparts occurs below 50 millosmolal, by which stage the temperature difference $(T_0 - T_A)$ is less than 0.015 °C in the former method (only 0.004 °C for the lowest solute concentration). Although the experimenter is spared the task of calculating O_A (or π) from the basic measurement, $(T_0 - T_A)$, there is need to keep in mind the magnitude of the temperature difference being manifested in the instrument response. This precaution is less critical for the measurement of osmoloality by freezing point depression because of the six-fold greater temperature difference for the same solution [Eqs. (2a) and (2b)]. It therefore seems probable that the disparity between the recorded measurements of osmolality in Fig. 1a merely reflects the difficulty of determining accurately the magnitudes of very small temperature differences between dewpoints.

Inspection of Fig. 1b shows that the osmolality range in question was not covered in the calibration plot (●) used to establish the concordance of results obtained by the two procedures for salt solutions [1]. On the grounds that the measurements for albumin solutions (Fig. 1a) essentially reflect the osmolality contributions of NaCl in the sample, those data (○) can be regarded as an extension of the calibration plot (Fig. 1b). The consequent indication of the unreliability of vapor pressure deficit measurements in the low osmolality range finds parallel in an earlier observation [3] that such values fail to extrapolate to zero solute concentration (as in Fig. 1a), and also in the report [4] that the response of vapor pressure deficit measurements is not linear below 50 milliosmolal. The reported disparity of results for aqueous serum albumin solutions [1] may thus reasonably be attributed to an unjustified extension of the comparison beyond the osmolality range for which the smaller temperature difference in vapor pressure deficit osmometry is of sufficient magnitude for accurate delineation of the osmotic potential.

3.2. Polyethylene glycol

Discrepancies between osmolality estimates were minimal for polyethylene glycols with relatively short chainlengths, but the values determined by freezing point depression were consistently and significantly higher for PEG 1000 and PEG 6000 [1]. Furthermore, the disparity persisted at polyethylene glycol concentrations for which uncertainty in the estimate of $(T_0 - T_A)$ could not have been an issue. The possibility therefore needs to be examined that the disparate experimental observations reflect the different temperatures to which the vapor pressure deficit and freezing point depression measurements apply (25 °C and 0 °C, respectively).

Confirmation of this interpretation of the Kiyosawa data [1] is provided by Fig. 2, which clearly signifies temperature dependence of the osmotic pressure of PEG 6000 that had been deduced by a single method–vapor pressure deficit osmometry [8]. Indeed, on the basis of such studies over the temperature range 15–35 °C, Michel [9] has formulated an empirical relationship,

$$(\pi)_P = 4.0\times10^{-4} w_A + (1.4\times10^{-3} - 1.29 \times 10^{-6} T) w_A^2 \quad (7)$$

in which $(\pi)_P$ signifies the predicted osmotic pressure (MPa) of a PEG 6000 solution with concentration w_A (g/kg) at temperature T (°C). Even though accuracy of the predictions of Eq. (7) is only claimed for temperatures within the range for which the empirical relationship was deduced, it transpires that Eq. (7) also describes

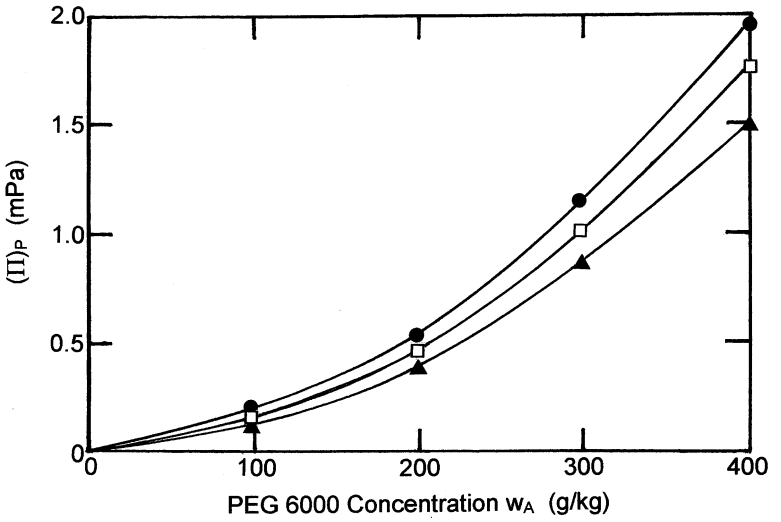

Fig. 2. Temperature and concentration dependence of the osmotic pressures of aqueous PEG 6000 solutions determined by the vapor pressure deficit method: ●, 15 °C; □, 25 °C; ▲, 35 °C. (Data taken from Ref. [8]).

reasonably well the osmolality data obtained by freezing point depression [1,3].

In Fig. 3, Eq. (6) is used to analyzed published results for PEG 6000 obtained by freezing point depression studies (○,□) as well as by vapor pressure deficit measurements at 25 °C (●,▲) and 70 °C (◆). Also, shown are the linear relationships predicted by Eq. (7) to account for the

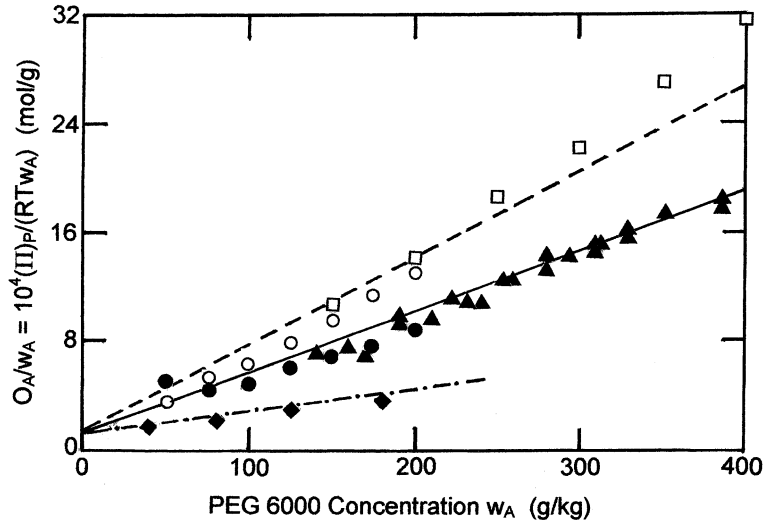

Fig. 3. Examination of results obtained by freezing point depression (○, □) as well as by vapor pressure deficit measurements at 25 °C (●,▲) and 70 °C (◆) in the light of the published temperature and concentration dependency of the osmotic pressure of aqueous PEG 6000 [9], the data being plotted according to Eq. (6). Lines signify the dependencies predicted by Eqs. (6) and (7) at 0 °C (-----), 25 °C (——) and 70 °C (-·-·-). Sources of data: ○,●, Ref. [1]; □, Ref. [3]; ▲, Ref. [9]; ◆, Ref. [2].

temperature dependence as well as concentration dependence of the osmotic pressure of PEG 6000. Eq. (7) not only describes the vapor pressure deficit data at 25 °C (●,▲) reported by Kiyosawa [1] and Michel [9], respectively, but also provides a reasonable description of the respective freezing point depression data (○,□) reported by Kiyosawa [1] and Steuter et al. [3]. Indeed, it also applies to the vapor pressure deficit measurements obtained by Rogers and Tam [2] at 70 °C (♦).

From the present viewpoint, the important feature of Fig. 3 is its provision of quantitative support for the contention that the discrepancy between osmolalities of PEG 6000 solutions deduced by freezing point depression and vapor pressure deficit osmometry does not signify a deficiency of either procedure. Instead, the difference reflects temperature dependence of the osmotic characteristics of polyethylene glycol. Consideration of the slopes in terms of Eq. (6) with π given by Eq. (7) gives rise to the following values of C_{AA} based on the currently accepted molar mass (M_A) of 8 kg for PEG 6000: 395 kg/mol at 0 °C; 278 kg/mol at 25 °C; and 105 kg/mol at 70 °C. On the basis of incorporating those values into Eq. (5), the molar second virial coefficient for self-interaction of PEG 6000 (B_{AA}) decreases progressively from 400 l/mol at 0 °C to 110 l/mol at 70 °C, it being 284 l/mol at the intermediate temperature (25 °C).

In statistical–mechanical terms, the parameter $2B_{AA}$ for an uncharged solute such as polyethylene glycol equates with the covolume for self-interaction (the volume from which the centers of two PEG molecules are mutually excluded). Although the conclusion that the effective size of PEG 6000 decreases with increasing temperature is at variance with the corresponding increase in molar volume [7], the two findings are not contradictory. Whereas the molar volume ($M_A\bar{v}_A$) deduced from density measurements [7] refers to anhydrous polyethylene glycol, B_{AA} refers to the solvated (hydrated) species. On the grounds that the chemical structure of polyethylene glycol is conducive to the formation of glycol–water hydrogen bonds, the hydration at 0 °C should be maximal and impart a degree of rigidity to the molecule. A decrease of excluded volume with increasing temperature then finds rational explanation in terms of (i) a decrease in the extent of solvation because of progressive hydrogen bond rupture (ΔH negative); (ii) a consequent increase in flexibility of the polymer chain; and (iii) a further reduction in size as the result of a greater contribution of hydrophobic interactions between backbone ($-CH_2CH_2-$) groups [7] to the energetics governing the conformation of the polyethylene glycol chains.

4. Summary

The purpose of this investigation has been to allay concerns about the validity of osmolality estimates deduced from vapor pressure deficit measurements; and thereby to highlight the fact that solutions to the dilemmas had been advanced long before their recent reincarnation. Isopiestic procedures such as vapor pressure deficit osmometry are clearly preferable to their freezing point depression counterpart because of their compatibility with determination of the osmotic characteristics at the temperature of interest rather than at the freezing point of solvent. For solutes such as simple sugars and salts the essential lack of temperature dependence of O_A renders irrelevant the method of its determination, be it vapor pressure deficit, freezing point depression, or boiling point elevation for that matter; but for polyethylene glycol the temperature of the measurement governs the osmolality of the solution.

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