

Osmotic Pressure of Aqueous Polyethylene Glycols¹

Relationship between Molecular Weight and Vapor Pressure Deficit

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ABSTRACT

Osmotic pressures (Π) of aqueous solutions of polyethylene glycols (PEGs) of average relative molecular weight (M_r) between 200 and 10,000 were measured using vapor pressure deficit osmometry. The relationships between molarity and Π were described with high precision by second order polynomials for each of the PEGs studied. In contrast to previous reports, equivalent weights of different polymers in solution did not generate the same Π ; low M_r PEGs generated a higher Π than the higher M_r PEGs. The effect of PEGs upon Π represents an interaction between concentration and M_r .

Water soluble PEGs have been widely used as inert, non-ionic solutes in the study of the water relations of plants (9, 16, 17), fungi (11, 13, 14), and animals (24). The availability of these polymers over a wide range of M_r has been exploited in measurements of cell wall porosity (3, 6, 13). Another important application has been the use of PEGs to promote the transformation and fusion of protoplasts (2, 7, 21).

In many experiments, it has been necessary to determine the osmotic pressure (Π^3) of PEG solutions. The Π has been measured using both freezing point depression and vapor pressure deficit osmometry (1, 5, 8, 12, 19, 20), and also by equilibration with sucrose solutions (10). However, much controversy persists concerning the best method for measuring the Π of polymer solutions and the relationships between concentration, M_r and Π (20).

Previous studies have employed molal PEG solutions (10, 12, 19, 20) which facilitates the thermodynamic interpretation of the relationship between concentration and Π by reference to the ideal gas equation (*i.e.*, molality corrects for solute volume) (4, 10). However, molarity is often a more convenient expression of concentration for experimental purposes and has been used in the present study. I have attempted to provide a set of standard curves relating molarity to Π for PEGs of average M_r between 200 and 10,000 over a range of concentrations encompassing those used in physiological experi-

ments. New information has also been provided on the relationship between M_r and Π .

Π was measured by vapor pressure deficit osmometry since the variation of osmotic coefficient (which accounts for the nonlinearity of concentration versus Π responses) is strongly temperature dependent (10, 12), reducing the usefulness of freezing point depression data at physiological temperatures (except for very dilute solutions).

MATERIALS AND METHODS

PEGs of average M_r between 200 and 10,000 were used as supplied by Fluka Chemical Corp., New York. The M_r ranges stated by the supplier are listed in Table I. For each concentration of each PEG, the solute was weighed out separately and dissolved in distilled water to avoid possible cumulative errors due to serial dilution. All measurements were made on fresh solutions at $21 \pm 1^\circ\text{C}$ within an hour of preparation to reduce error due to evaporative concentration. Ten μL samples were pipetted onto 6 mm diameter filter paper discs placed previously into the sample chamber of a Wescor 5100C Vapor Pressure Deficit Osmometer (Wescor, Inc., Logan, UT). The osmolality of each solution was measured repeatedly until a series of three readings were obtained which lay within a 5 mosmol range (minimum of three replicates per concentration of each PEG). A mean value of osmolality was calculated from the closest three measurements and the solvent contribution (correction factor = osmolality of distilled water registered by the osmometer) was subtracted from the mean. The correction factor was determined at the end of each concentration of PEG to adjust for osmometer drift due to thermocouple contamination (22). The osmometer was frequently recalibrated over the range 100 to 1000 $\text{mmol}\cdot\text{kg}^{-1}$ using NaCl osmolality standards (Wescor, Inc.) during the experiments.

Osmolality measurements were converted to Π (MPa) using the formula $\Pi = RTc$; where c = osmolality in $\text{moles}\cdot\text{kg}^{-1}$ and $RT = 2.446 \text{ kg}\cdot\text{MPa}\cdot\text{mol}^{-1}$ at 21°C .

RESULTS AND DISCUSSION

Figure 1 shows the relationships between molarity and Π for PEG-200 through -10,000. As reported in previous investigations (5, 10, 20), there was not a linear relationship between concentration and Π . This behavior reflects considerable divergence from ideality (ideal behavior of KCl is shown in Fig. 1A for comparison). However, the relationships be-

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³ Abbreviation: Π , osmotic pressure.

tween molarity and Π were described with a high degree of precision by second order polynomials for the complete range of PEGs studied (Table IIA). Curves were fitted by regression analysis and F values were all significant at $P \leq 0.0001$.

PEG concentration is often expressed as a percentage, sometimes without qualification as either w/v solution (molar) or w/w solvent (molal) (24, 25). However, the qualification is an important one since molar solutions (being more concentrated) increase the Π to a greater extent than molal solutions of the same numerical value. This is shown in Figure 2 for the most commonly used PEG with an average M_r of 6000. Within the resolution of the osmometer (~ 5 mosmol), this effect was exhibited above 10 mmolar/10 mmolal ($60 \text{ g} \cdot \text{L}^{-1}$ solution or $60 \text{ g} \cdot \text{kg}^{-1}$ distilled water) as the molar volume of the polymer began to affect the concentration of the solution significantly.

For a given molarity, the larger polymers clearly increased the Π more than the smaller ones (Fig. 1). This effect of M_r on Π has also been documented, based upon osmolality determinations of molal solutions, by Steuter *et al.* (20) who suggested that "... the total mass or total number of molecular subunits, rather than total number of particles (polymer chains) may be an important factor controlling water potential." The data of Williams and Shaykewich (23) for PEG-6000 and $\sim 20,000$ were in agreement with this conclusion. In the present study, a comparison was made between solutions of identical volume containing identical weights of the different polymers (*i.e.*, a range of molarities). Figure 3 shows that low M_r PEGs had a greater effect upon Π than higher M_r s. The effect of these polymers upon Π represents a more complex interaction between concentration and M_r than that suggested by Steuter *et al.* (20); although the mass of material in solution does have a strong influence upon the vapor pressure (Fig. 1), long chain PEGs do not behave as a number of individual subunits (Fig. 3). Previous studies (20, 23) were limited to the higher M_r PEGs obscuring this strong influence of polymer size on Π .

In common with the PEGs, sucrose did not behave as an ideal solute (Fig. 1A). However, sucrose generated a lower Π than that predicted for a PEG of comparable M_r (Fig. 3). This suggests that the effect of PEGs upon Π reflects specific characteristics of these polymers. There have been various proposals to account for the deviation from ideality of PEGs. On the basis of viscosity measurements, Michel and Kauf-

Table I. Average M_r of PEGs Used in the Study with Corresponding M_r Ranges Determined by the Supplier (Fluka Chemical Corp.)

Average M_r	M_r Range
200	190–210
300	285–315
400	380–420
600	570–630
1,000	950–1,050
1,500	1,400–1,600
2,000	1,900–2,200
3,000	2,700–3,300
4,000	3,500–4,500
6,000	6,000–7,500
10,000	8,500–11,500

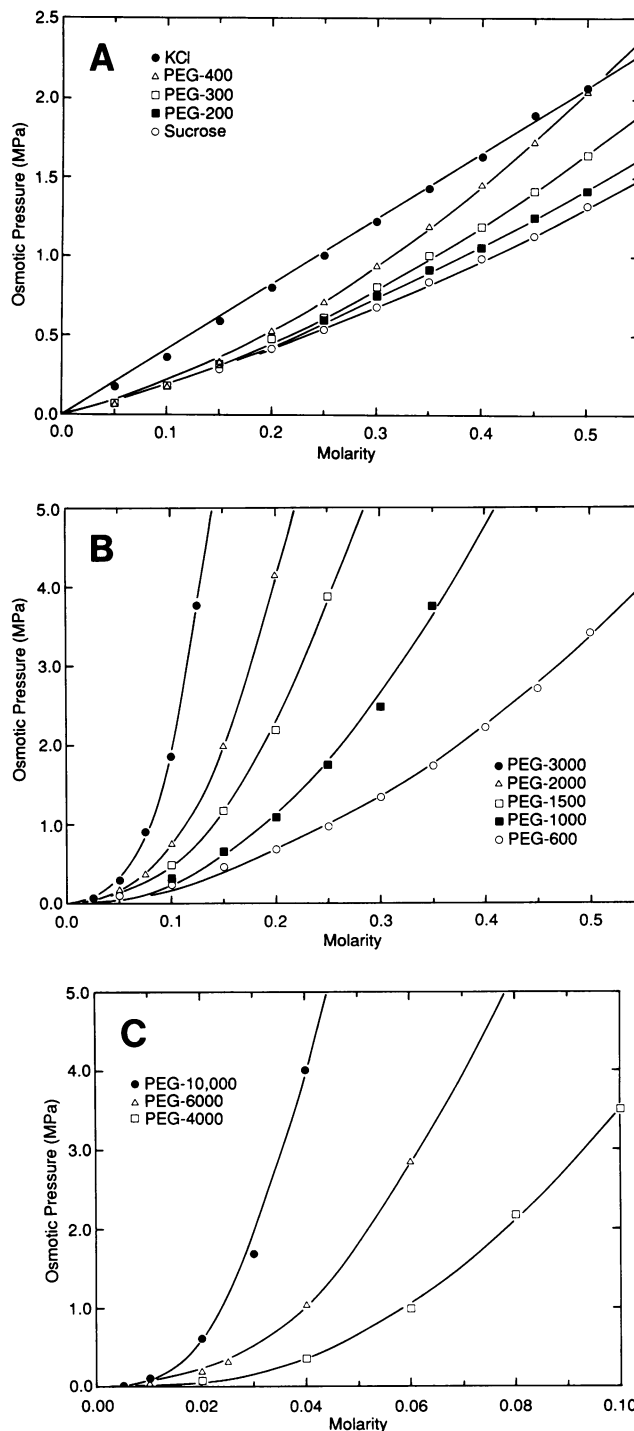


Figure 1. Relationships between concentration (molarity) and osmotic pressure (Π) for PEG-200 through -10,000, sucrose, and KCl. Note the changes in scale of abscissa and ordinate between A, B, and C. Curves were fitted by regression analysis; see Table IIA.

mann (12) suggested that the configuration of the PEG molecule might change in response to concentration: extended at low concentrations, folding with increasing concentration. PEG-6000 in an aqueous solution exists as rigid helical segments and most of the hydrogen bonding of water occurs at those oxygens exposed at positions along the disordered parts

Table II. Curves Fitted by Regression Analysis

Degrees of freedom (df) are for model, error

A. Curves in Figure 1 relating Π to concentration were fitted by polynomial regression and constrained to pass through zero. Accuracy limited at very low solute concentrations.Form: $\Pi = \alpha \cdot C + \beta \cdot C^2$, where Π is osmotic pressure in MPa and C is the molarity.

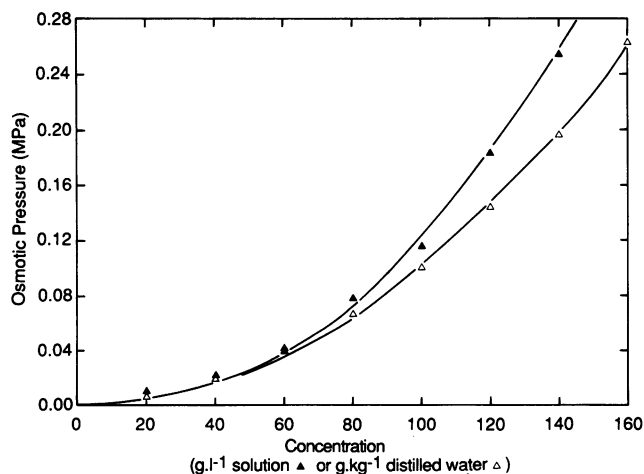
M_r^a	α	β	r^2	F	P	df
200	1.9	1.8	0.9995	11,106	0.0001	2, 10
300	1.7	3.3	0.9998	30,243	0.0001	2, 10
400	1.6	5.0	0.9998	23,495	0.0001	2, 10
600	1.0	11.4	0.9997	18,279	0.0001	2, 10
1000	-0.8	31.9	0.9980	1,494	0.0001	2, 6
1500	-2.9	72.3	0.9986	1,448	0.0001	2, 4
2000	-5.2	128.1	0.9986	1,391	0.0001	2, 4
3000	-12.1	328.4	0.9940	334	0.0001	2, 4
4000	-8.5	435.2	0.9992	2,542	0.0001	2, 4
6000	-12.1	980.0	0.9990	2,019	0.0001	2, 4
10000	-37.9	3379.3	0.9947	378	0.0001	2, 4
SUC ^b	1.7	1.9	0.9997	15,594	0.0001	2, 10
KCl ^b	4.1		0.9997	34,021	0.0001	2, 11

B. Curves in Figure 2, relating concentration of PEG-6000 ($\text{g} \cdot \text{L}^{-1}$ solution and $\text{g} \cdot \text{kg}^{-1}$ H_2O) to Π (MPa) fitted by polynomial regression as above (same form of equation as A).

Concn.	α	β	r^2	F	P	df
$\text{g} \cdot \text{L}^{-1}$ (molar)	-2.7×10^{-4}	1.5×10^{-5}	0.9985	2260	0.0001	2, 7
$\text{g} \cdot \text{kg}^{-1}$ (molal)	5.1×10^{-5}	9.8×10^{-6}	0.9996	8313	0.0001	2, 7

C. Curves of Figure 3, relating M_r to Π (MPa) fitted by regression.Form: $\Pi = \gamma (\log M_r)^\delta$, where Π is the osmotic pressure (MPa) and M_r = the average mol wt.

Concn.	γ	δ	r^2	F	P	df
$300 \text{ g} \cdot \text{L}^{-1}$	41.9	-2.4	0.9185	101	0.0001	1, 9
$200 \text{ g} \cdot \text{L}^{-1}$	54.6	-3.4	0.9483	165	0.0001	1, 9
$100 \text{ g} \cdot \text{L}^{-1}$	540.7	-7.1	0.9411	112	0.0001	1, 7

^a M_r values refer to PEGs. ^b Sucrose (SUC) and plot for KCl is linear ($\beta = 0$).**Figure 2.** Relationship between concentration and osmotic pressure for PEG-6000, molar and molal series. Curves fitted by regression; see Table IIB.

of the helix (unpublished data of GJ Safford discussed in ref. 12). Disorder and binding of water could increase with concentration, depressing the thermodynamic activity of water and increasing the measured Π . Steuter *et al.* (20) argued for a micellar or colloidal behavior of PEGs in solution, and a disproportionate effect upon the chemical potential of water due to resulting matric effects. The Π measured by vapor pressure deficit osmometry integrates both the dissolved solute and matric components (15, 18), which influence the vapor pressure above the sample. Whether the matric component of Π is significant or not, vapor pressure deficit data represent the best available guide that we have to the total osmotic effect of PEGs in solution. PEGs can be used effectively when elevated ionic or metabolizable solutes are undesirable in an experiment.

It should be noted that there is some synergism between ionic components and PEGs when they are combined in solution (8, 12); simple addition of the separate values of Π for the unsupplemented nutrient solution and for the PEG in distilled water may not reflect the absolute Π of the medium. The Π of a complex medium supplemented with PEG can be more than 7% higher than the value predicted by addition of the separate Π s of the different components (12) (NP Money,

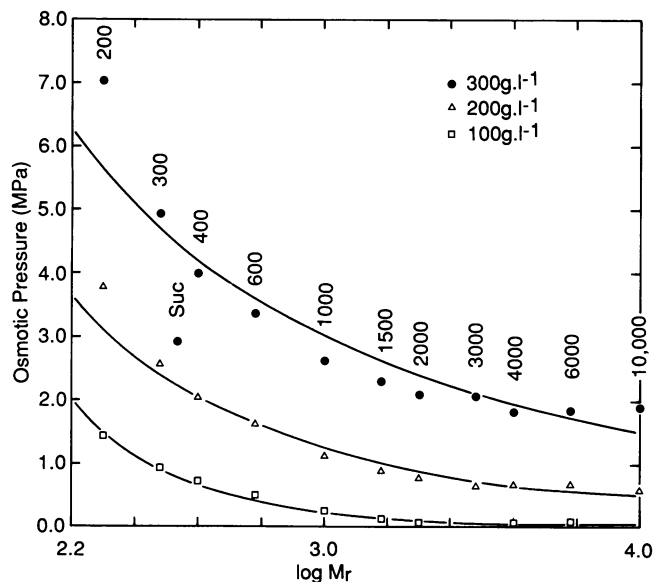


Figure 3. Relationship between average M_r and osmotic pressure (Π) for three different concentrations of polymer. Sucrose ($300\text{ g}\cdot\text{L}^{-1}$ only) shown for reference. Data points derived from curves fitted to measurements of Figure 1, by interpolation and extrapolation. Curves fitted by regression; see Table IIC.

unpublished observations). Therefore, when the value of Π is of critical importance, osmometric determinations should be made upon PEGs in the experimental medium.

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