

Letters to the Editor

An Improved Fit to Website Osmotic Pressure Data

Osmotic pressure has become a useful perturbant of biophysical and biochemical systems. The addition of inert osmolyte to any aqueous system stabilizes molecular configurations of low osmolyte-excluded volume; i.e., the system is placed under “osmotic stress.” By this method, it has been possible to generate compacting forces on apposed membranes (LeNeveu et al., 1976) and on DNA condensates (Podgornik et al., 1995), to determine changes of channel volumes during gating (Zimmerberg et al., 1990), and to measure volume and hydration changes associated with protein function (Rand et al., 1993; Reid and Rand, 1997), protein binding to nucleic acids (Garner and Rau, 1995), and protein binding to other proteins (Highsmith et al., 1996).

For systems consisting of two states in equilibrium, osmotic pressure shifts the equilibrium toward the state of lower osmolyte-inaccessible volume via a $\Pi\Delta V$ term in the free energy ΔG , where Π is the osmotic pressure and ΔV is the difference in osmolyte-inaccessible volume between the two states. The product $\Pi\Delta V$ is obtained from the shift of equilibrium observed upon addition of osmolyte to the bulk aqueous phase. To obtain ΔV , which is generally the parameter of physical interest, Π must be known. To obtain Π , it is common to refer to previously measured data of osmotic pressure versus concentration (in wt% = g/dl) for the osmolyte of interest.

Π versus $C_{\text{wt}\%}$ data for various osmolytes are available in the literature (LeNeveu et al., 1976; Parsegian et al., 1986, 1995), and excellent, up-to-the-minute compilations of useful data are now available on the World Wide Web (Website 1, Selected osmotic stress data, <http://www.mgsl.dcrn.nih.gov/docs/osmdata/osmdata.html>; Website 2, Osmotic pressure data, <http://aqueous.labs.brocku.ca/osfile.html>). Data from these sources have been cited in this journal (Rau and Parsegian, 1992; Vodyanoy et al., 1993; Coorsen and Rand, 1995; Highsmith et al., 1996). The Website data, in most cases, are tabulated as $\log \Pi$ versus $C_{\text{wt}\%}$, with Π in dyne/cm². On the Websites, as done previously by Parsegian et al. (1986, 1995), the empirical expression

$$\log \Pi = a + b(C_{\text{wt}\%})^c \quad (1)$$

is fitted to the data for each osmolyte to provide continuous values of Π over the measured range. Fitted values of the arbitrary parameters a , b , c from Eq. 1 are then given for each osmolyte. This expression is intended to be restricted to the data range; i.e., it is not reliable for extrapolation above or below the measured points, as cautioned by the

authors (Website 1, Website 2). It is clear, for example, that Eq. 1 does not give the correct limiting value of Π (which is zero) as $C_{\text{wt}\%}$ goes to zero. Recently other fits to the Website data have also been proposed (Reid and Rand, 1997).

In this letter we give reasons for using a polynomial expression for Π as a function of $C_{\text{wt}\%}$ in place of Eq. 1. The well-established polynomial expression for analysis of osmotic pressure data (Flory, 1953; Tanford, 1961) has the following advantages: 1) it has a physical basis; 2) it gives the correct limiting behavior at low Π ; 3) it permits use of the osmolyte molecular weight, if known, in fitting Π versus $C_{\text{wt}\%}$ data, ensuring accurate extrapolation to arbitrarily low values of Π ; 4) if the osmolyte molecular weight is not known, it provides a method for extrapolating Π versus $C_{\text{wt}\%}$ data to low Π and for determining the molecular weight; 5) it yields information on osmolyte excluded volumes and osmolyte-solvent and osmolyte-osmolyte interactions; 6) it provides useful identification of questionable data points, especially at low Π .

The polynomial expression derives from the virial expansion for Π as a function of osmolyte concentration:

$$\Pi = RT(c + ac^2 + bc^3 + \dots) \quad (2)$$

where c is in molar units, and a and b are constants. At low c , Eq. 2 reduces to van't Hoff's limiting law, i.e., $\Pi(c)$ goes linearly to zero with slope RT . In terms of $C_{\text{wt}\%}$, $c = (10/M)C_{\text{wt}\%}$, where M is the number-average molecular weight of the osmolyte in Daltons (g/mol). Substituting this expression in Eq. 2 gives

$$\Pi = RT \left[\frac{10}{M} C_{\text{wt}\%} + AC_{\text{wt}\%}^2 + BC_{\text{wt}\%}^3 + \dots \right] \quad (3)$$

where $A = a(10/M)^2$ and $B = b(10/M)^3$. A and B are the second and third virial coefficients, respectively. Dividing Eq. 3 by $C_{\text{wt}\%}$,

$$\frac{\Pi}{C_{\text{wt}\%}} = RT \left[\frac{10}{M} + AC_{\text{wt}\%} + BC_{\text{wt}\%}^2 + \dots \right] \quad (4)$$

For small $C_{\text{wt}\%}$ ($C_{\text{wt}\%} \ll A/B$), Eq. 4 reduces to

$$\frac{\Pi}{C_{\text{wt}\%}} = RT \left[\frac{10}{M} + AC_{\text{wt}\%} \right] \quad (5)$$

Thus at low $C_{\text{wt}\%}$, a plot of $\Pi/C_{\text{wt}\%}$ versus $C_{\text{wt}\%}$ is linear, with the y intercept determined by M and the slope determined by A . When M is independently known, Eq. 3 gives a constrained third-order polynomial expression for $\Pi(C_{\text{wt}\%})$ with two arbitrary parameters, A and B , if higher-order terms are neglected. Equivalently, Eq. 4 gives a constrained second-order polynomial expression with the same parameters A and B .

We illustrate here a typical case, polyethylene glycol, $M = 300$ Da (PEG300). In Fig. 1 A the data for PEG300 (Website 2) are plotted in the form Π versus $C_{wt\%}$. The two-parameter constrained polynomial (solid line, Eq. 3) fits the data in the measured range better than does the three-parameter logarithmic expression (dotted line, Eq. 1). Inclusion of the point $\Pi = 0$ at $C_{wt\%} = 0$ (open square) along with the use of M to define the initial slope $= 10 RT/M$ constrains the fit and allows accurate values of Π to be obtained at low $C_{wt\%}$, below the measured range. Such values should be useful to workers in the field.

The utility of the polynomial fit is made clearer when the data are plotted as $\Pi/C_{wt\%}$ versus $C_{wt\%}$ (Eq. 4), shown in Fig. 1 B. By use of M , the y intercept $= 10 RT/M$ is

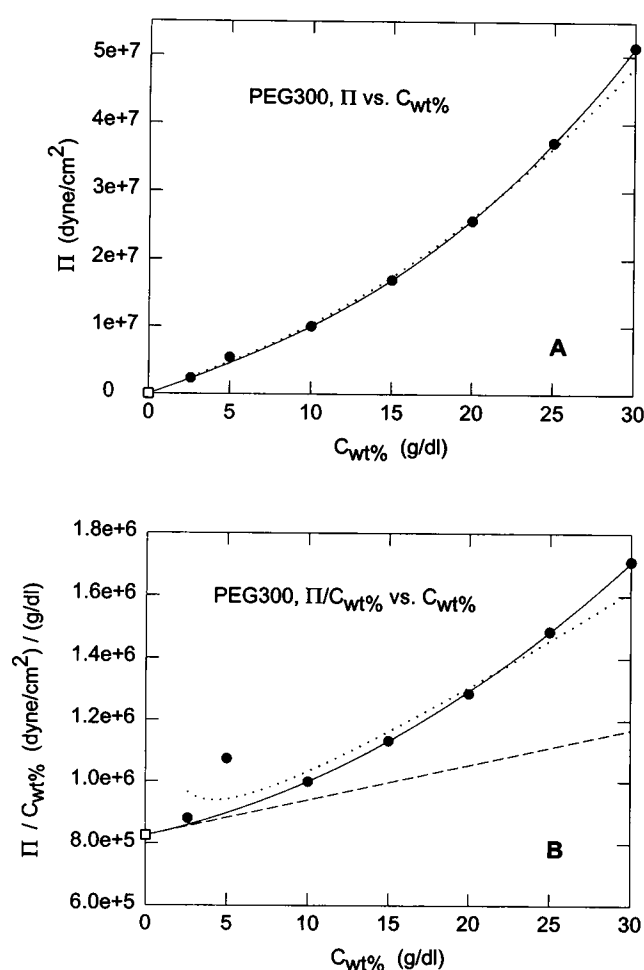


FIGURE 1 PEG300 data and fits. ●, Data from Website 2. (A) Π versus $C_{wt\%}$ plot. —, Two-parameter constrained polynomial fit (Eq. 3), using $M = 300$ Da and the fitted parameters $RTA = 1.14 \times 10^4$, $RTB = 598$, in units defined by Eq. 3. □, Point at $(\Pi, C_{wt\%}) = (0, 0)$ used in the polynomial fit. ·····, Website fit (Eq. 1) with $a = 4.79$, $b = 1.28$, $c = 0.24$. (B) $\Pi/C_{wt\%}$ versus $C_{wt\%}$ plot. —, Two-parameter constrained polynomial fit (Eq. 4) using the same parameters as in A. ---, Linear term of polynomial fit (Eq. 5). □, y intercept $= 10 RT/M$, using $M = 300$ daltons and $RT = 2.48 \times 10^7$ (dyne/cm²)/(mol/liter) at 25°C. ·····, Website fit (Eq. 1) with a, b, c as in A. The data point at $C_{wt\%} = 5\%$ was excluded from the polynomial fits.

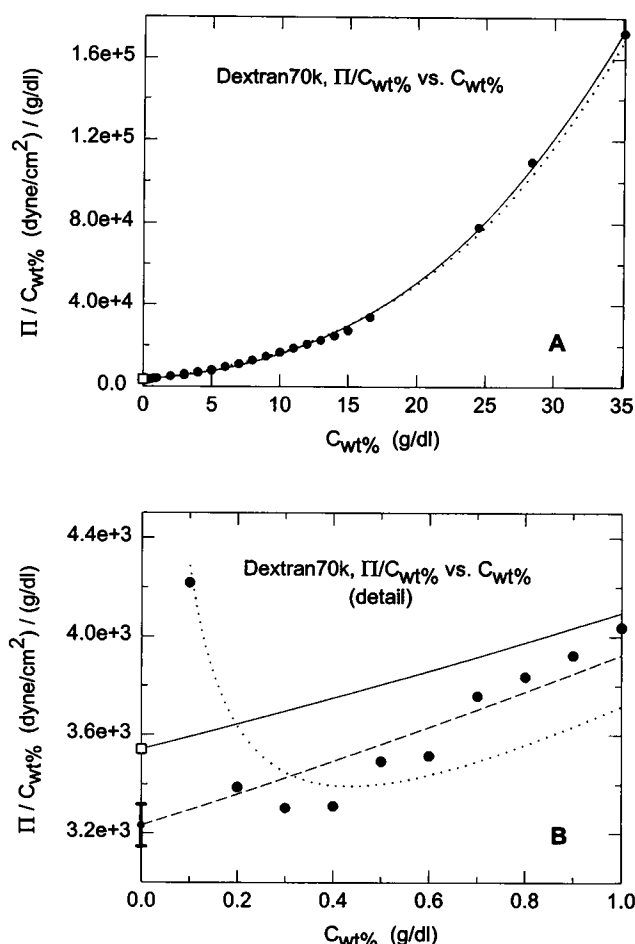


FIGURE 2 Dextran T110, $M = 70$ kDa, data and fits. (A) $\Pi/C_{wt\%}$ versus $C_{wt\%}$ plot. ●, Data from Website 1. — and □, Three-parameter constrained polynomial fit and y intercept $= 10 RT/M$ (Eq. 4), using the nominal $M = 70$ kDa with fitted parameters $RTA = 499.9$, $RTB = 52.95$, $RTC = 2.021$, in units defined by Eq. 3. This fit includes the fourth virial coefficient in Eq. 4. ·····, Six-parameter Website fit (Eq. 1) with $a = 1.385$, $b = 2.185$, $c = 0.2436$ for $C_{wt\%} < 10\%$, and $a = 1.872$, $b = 1.657$, $c = 0.3048$ for $C_{wt\%} > 10\%$. (B) Detail of A for $C_{wt\%} \leq 1\%$. ●, □, —, ·····, are the same as in A. ---, Three-parameter unconstrained polynomial fit (Eq. 4) to data up to $C_{wt\%} = 10\%$. The fitted parameters are $10 RT/M = 3232$, $RTA = 621.7$, $RTB = 72.21$, in units defined by Eq. 3. The y intercept (dot with standard-error bar) yields $M = 77 \pm 2$ kDa. A four-parameter unconstrained polynomial fit to all of the data up to 35.1% yields a less precise $M = 69 \pm 7$ kDa (not shown). The anomalous data point at $C_{wt\%} = 0.1\%$ was excluded from the polynomial fits. The non-physical behavior of the Website fit at low $C_{wt\%}$, seen here and in Fig. 1 B, is not data dependent but is an intrinsic feature of Eq. 1.

determined (indicated by the open square). It is apparent from this plot that the data point at 5 wt% is anomalous. The irregularity of this point is barely evident in the Π versus $C_{wt\%}$ plot (Fig. 1 A), and it is even less discernible in a log Π versus $C_{wt\%}$ plot. An advantage of $\Pi/C_{wt\%}$ versus $C_{wt\%}$ plots is their emphasis on the quality of the data near $\Pi = 0$ and their exposure of aberrant data points.

For an unknown M , Eq. 4 can be fitted to data of $\Pi/C_{wt\%}$ versus $C_{wt\%}$ as a three-parameter, unconstrained second-order polynomial, yielding M from the y intercept =

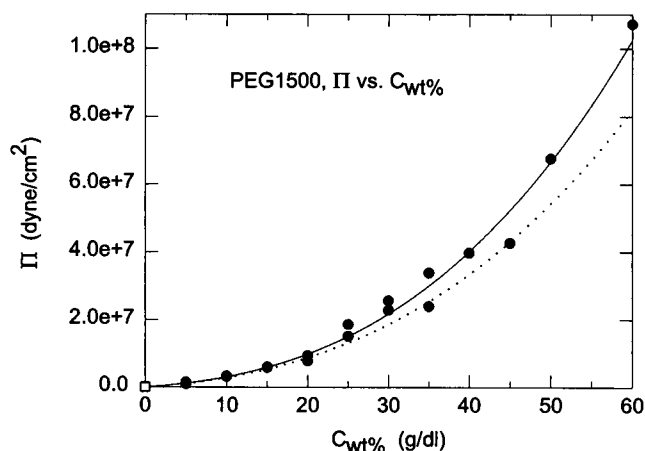


FIGURE 3 PEG1500 data and fits; Π versus $C_{wt\%}$ plot. ●, Data from Website 2. —, Two-parameter constrained polynomial fit (Eq. 3), using $M = 1500$ Da with fitted parameters $RTA = 1.16 \times 10^4$, $RTB = 235$, in units defined by Eq. 3. □, Point at $(\Pi, C_{wt\%}) = (0, 0)$ used in the polynomial fit. ····, Website fit (Eq. 1) with $a = 3.72$, $b = 1.57$, $c = 0.24$.

TABLE 1 PEG second (A) and third (B) virial coefficients determined by polynomial fits to Website 2 osmotic pressure data

PEG M , nominal (Da)	A	B
300	$(4.6 \pm 0.3) \times 10^{-4}$	$(2.4 \pm 0.1) \times 10^{-5}$
400	$(5.6 \pm 1.0) \times 10^{-4}$	$(2.0 \pm 0.4) \times 10^{-5}$
600	$(-4.6 \pm 1.7) \times 10^{-4}$	$(4.2 \pm 0.3) \times 10^{-5}$
1000	$(7.6 \pm 0.9) \times 10^{-4}$	$(0.3 \pm 0.2) \times 10^{-5}$
1500	$(4.7 \pm 1.0) \times 10^{-4}$	$(0.9 \pm 0.2) \times 10^{-5}$
2000	$(2.4 \pm 0.7) \times 10^{-4}$	$(1.8 \pm 0.2) \times 10^{-5}$
3000	$(1.7 \pm 0.4) \times 10^{-4}$	$(1.9 \pm 0.1) \times 10^{-5}$
4000	$(5.4 \pm 1.0) \times 10^{-4}$	$(0.6 \pm 0.3) \times 10^{-5}$
6000	$(2.0 \pm 0.7) \times 10^{-4}$	$(1.8 \pm 0.2) \times 10^{-5}$
8000	$(2.1 \pm 1.1) \times 10^{-4}$	$(1.7 \pm 0.4) \times 10^{-5}$
10000	$(1.4 \pm 0.7) \times 10^{-4}$	$(2.0 \pm 0.2) \times 10^{-5}$
20000	$(2.1 \pm 0.2) \times 10^{-4}$	$(1.47 \pm 0.07) \times 10^{-5}$

A and B were determined from two-parameter constrained polynomial fits of Eq. 4 to the data, using the nominal value of M in each case. The units of A and B are defined by Eq. 3. The uncertainties are standard errors.

10 RT/M . When this is done for the data in Fig. 1 B (excluding the anomalous point at 5 wt%), the result is $M = 290 \pm 1$ Da, which is undoubtedly more accurate than the nominal $M = 300$ Da. However, $\Pi/C_{wt\%}$ versus $C_{wt\%}$ plots of the Website data reveal that in general there are too few data points of sufficient precision at low $C_{wt\%}$ to permit accurate determinations of M . Although the unconstrained polynomial Eq. 4 uses all of the data to determine M , the data at low $C_{wt\%}$ are clearly the most important. One criterion for a reliable M determination is that for $C_{wt\%} \leq 0.1(A/B)$, the data lie on a straight line as prescribed by Eq. 5. For PEG300 this range is $C_{wt\%} \leq 2\%$ (cf. Fig. 1 B, dashed line).

Data were reported between $C_{wt\%} = 0.1\%$ and 35.1% for dextran T110, $M = 70$ kDa (Website 1). The Website fit to these data (Eq. 1) uses six fitting parameters: a , b , c for $C_{wt\%} < 10\%$ and a different a , b , c for $C_{wt\%} > 10\%$. By

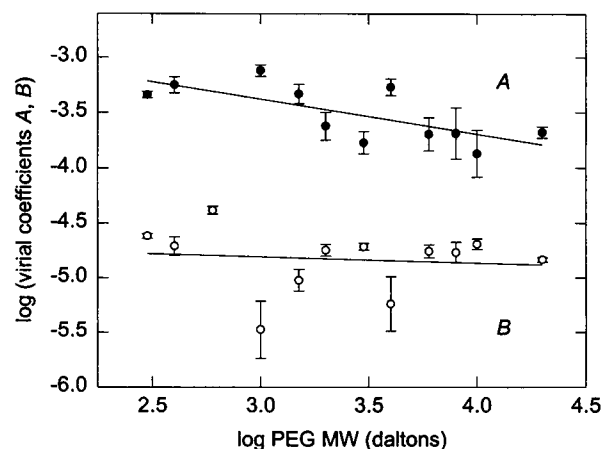


FIGURE 4 Log-log plots of the second (A) and third (B) virial coefficients versus nominal PEG molecular weight. A and B were determined from two-parameter constrained polynomial fits (Eq. 4) to the Website data. ●, log A; ○, log B; bars indicate standard errors of the fitted parameters A and B. —, Best linear fits to the log-log plots; log A, slope = -0.32 ± 0.10 , y intercept = -2.4 ± 0.4 ; log B, slope = -0.06 ± 0.16 , y intercept = -4.6 ± 0.5 . No log A point is shown for $M = 600$ Da, as the fitted value of A is negative. The units of A and B are defined by Eq. 3.

comparison, a single three-parameter constrained polynomial (Eq. 3 or Eq. 4, including the fourth virial coefficient) is able to fit these data over the entire measured range. Fig. 2 A shows a $\Pi/C_{wt\%}$ versus $C_{wt\%}$ plot of the data, the constrained polynomial fit using the nominal value of M (solid line, Eq. 4, including a $C_{wt\%}^3$ term), and the Website logarithmic fit (dotted line, Eq. 1, with separate fits above and below $C_{wt\%} = 10\%$). Fig. 2 B shows a detail of these plots below $C_{wt\%} = 1\%$. Also shown is an unconstrained polynomial fit to the low $C_{wt\%}$ data for evaluation of M (dashed line, Eq. 4). The y intercept gives $M = 77 \pm 2$ kDa, indicating that the nominal M is only approximately correct. Fig. 2 B illustrates the very stringent requirements for accurate M determinations from osmotic pressure data.

When polynomial fits are done for all of the PEG data available on Website 2, with M ranging from 300 to 20,000 Da and $C_{wt\%}$ ranging from 1.5% to 67.5%, we find that the two-parameter constrained polynomials (Eqs. 3 and 4) fit the data well, and that higher-order terms are not necessary. An example of PEG1500, measured up to $C_{wt\%} = 60\%$, is shown in Fig. 3.

The virial coefficients A and B in Eqs. 3 and 4 contain information on osmolyte excluded volumes, osmolyte-solvent interactions, and osmolyte-osmolyte interactions. Table 1 gives A and B, determined from fits of Eq. 4 to all of the PEG data of Website 2. In Fig. 4, log A and log B are plotted versus log M (nominal). The slope of log A versus log M is close to the value -0.2 expected for polymers in good solvents (Tanford, 1961; de Gennes, 1979).

In conclusion, we point out that polynomial fits to osmotic pressure data have many advantages over fits such as Eq. 1.

We thank Prof. Edwin Thomas for an enlightening discussion of the M dependence of virial coefficients.

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Fits to Osmotic Pressure Data

Cohen and Highsmith have provided alternative, polynomial fits to empirically measured osmotic pressures of solutions, particularly those available for general use in osmotic stress experiments (Parsegian et al., 1995). There are several ways such data can be fit. We (Reid and Rand, 1997) have suggested another alternative to what was published earlier (Parsegian et al., 1995), based on the original idea of Haldane (1928) and recently used by Fullerton et al. (1992). According to that simple idea, nonideal osmotic pressure results because of the “binding” of water to solute, effectively removing it from activity, or equivalently, because of the exclusion of neighboring solute molecules from each solute molecule’s local “hydration shell.” Such mutual exclusion, or preferential hydration, is the basis behind the use of “osmotic stress” to probe aqueous compartments.

In addition to such “binding” or exclusion, it is observed (see Table 1 and Fig. 1) that osmotic pressures of equivalent weight concentrations of larger solutes become independent of molecular weight. The osmotic pressures of polyethylene glycols (PEGs) become practically independent of their nominal osmolality, as determined from their nominal or number average, or “vendor MW” (MW_{vendor}). The larger molecules behave osmotically as if they have a considerably smaller ideal or effective molecular weight (MW_{eff}), or equivalently, they behave colligatively, as if the osmotic unit were a fraction of a molecule. This is consistent with the idea that for very large polymers, as with persistence lengths, some effective mass acts osmotically independently of distant parts of the molecule.

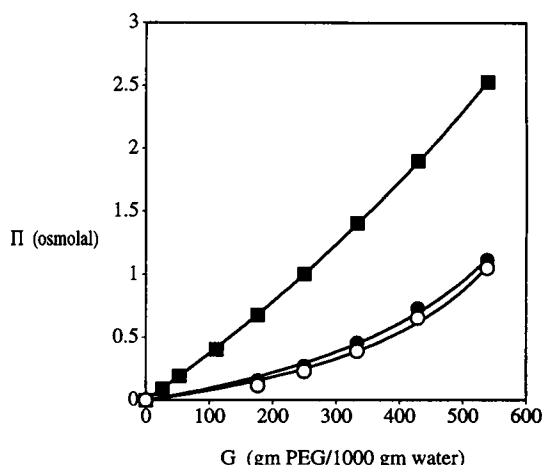


FIGURE 1 Plot of the empirical data, Π versus G , as shown in Table 1, for PEGs of MW_{vendor} 300 (■), 3000 (●), and 8000 (○). Osmotic pressure is nearly independent of MW_{vendor} for higher MWs. The fitted lines are to $\Pi = (G/MW_{\text{eff}})/1000 - m^*(G)$; the fitting parameters MW_{eff} and m are provided in Table 1.

The table provides our complete set of new experimental data for 10 different PEGs. These data are entirely consistent with our older data, used by Cohen and Highsmith. These data will be integrated with the older data, and the alternative fits discussed in these letters will be made available at the websites <http://aqueous.labs.brocku.ca/osfile.html> and <http://www.mgsi.dcrn.nih.gov/docs/OsmoticStress.html>.

Our fits to nonideal osmotic pressure were made on the following basis: 1) that m grams of water per gram of PEG are effectively removed by PEG from being active, or exclude other PEG molecules (following Haldane, 1928);