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Experimental compressibilities and average intermolecular distances of poly(ethylene glycol) molecular masses 2000–8000 Da in aqueous solution

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ABSTRACT

Small-Angle Neutron Scattering (SANS) is a powerful, nondestructive technique that can measure simultaneously macroscopic compressibilities of solutions and overall shapes of macromolecular solutes, as well as their intermolecular structural correlations. We have conducted SANS experiments on aqueous solutions of polyethylene glycols (PEGs) with nominal molecular masses 2000, 4000, and 8000 Da over the q-range 0.03–0.30 Å^{-1} [$q = (2\pi/\lambda)\sin\theta$]. By incorporating accurate background subtraction and short extrapolations of the intermolecular structure factor $S(q)$ down to $q = 0$, the isothermal compressibility can be measured. The results indicate a significant and systematic dependence of the solutions' compressibility on both molecular mass and concentration of PEG, unlike the solutions' osmotic pressures and activity of the water. This implies that the structure of water in the vicinity of PEG is considerably altered relative to the bulk state even though the activity coefficient of water remains nearly invariant in this range. Graphs of $S(q)$ for 3% w/w to 17% w/w solutions each show a gradual rise from the low-q side to a broad plateau, which indicates weak intermediate-range correlations between oligomers that are probably associated with soft, repulsive, solvent-mediated PEG–PEG interactions. Since both the water and PEG change structures from their neat forms, any quantitative assignment of changes in partial volumes must necessarily be arbitrary. However, the linear change in compressibility with PEG concentration below ~7% w/v can be said to indicate a composite solution, which parallels the behavior of composite solids.

1. Introduction

Proteins and polymers undoubtedly interact in solution, and these interactions can be manipulated, *inter alia*, to improve protein crystallizations for X-ray structure work [1]. The polymer most often used to affect these crystallizations is poly(ethylene glycol), PEG [1]. Measures of the extent of these interactions include second virial coefficients as well as decreases in protein solubility upon addition of PEGs or a different protein to the buffered, saline solutions. The beneficial interactions cannot result from simple mass action principles. In fact, the added PEGs can have more influence than adding an equal mass of protein. While investigating the effects of PEGs on protein crystallization, we found that the PEGs alone possessed the unexpected properties that are presented here.

Properties of aqueous solutions of PEG with molecular masses from the millions to diethylene glycol have been studied by a wide variety of techniques. Here, we limit our study to the molecular masses used in aiding protein crystallization: nominal molecular masses from 2000 to 8000 Da. Each of the PEGs is composed of a homologous series of oligomers.

The primary experimental tool for this study is Small-Angle Neutron Scattering (SANS) from PEG solutions in D_2O. From this scattering measured on a series of PEG concentrations, the average distance between these scatterers can be calculated. Also, from an extrapolation of the data, the bulk solution compressibilities relative to that of the pure solvent can be found (e.g., Rubinson [2]).

The bulk compressibility measured for pure liquids and for solutions has the same units. Even so, the measure for solutions is usually (and confusingly) called the osmotic compressibility. The bulk compressibilities of pure solvents and their solutions can be related through Equation (1), as originally presented by Dijkstra et al. [3]. The bulk volume compressibility is $\chi_T = -V^{-1}(\partial V/\partial p)$. The solution's compressibility is $\chi_{T,\mathrm{eff}} = -V^{-1}(\partial V/\partial \pi)_{n_1,z_2}$ where n_1 is the number of moles of solute, z_2 is the fugacity of the solvent,

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here water (D_2O), and the osmotic pressure π substitutes for its equivalent solute concentration. The two compressibilities are related [3] by

$$\chi_T^{-1} - \chi_{T,\mathrm{eff}}^{-1} = -V\left(\frac{\partial z_2}{\partial V}\right)_{n_1,n_2}\left[\left(\frac{\partial \pi}{\partial z_2}\right)_{n_1,V} + \left(\frac{\partial p_2}{\partial z_2}\right)\right] \quad (1)$$

In Equation (1), subscript 1 refers to the solute and 2 refers to the solvent. As noted by Dijkstra, the second term in square brackets—the variation of the pressure of the solvent with respect to its fugacity—usually is much smaller than the first and can be ignored. This is also true for the PEG solutions here.

The commonly accepted idea that a small volume change is accompanied by a large compressibility change is explained quantitatively through the volume dependence of the solvent's fugacity. SANS can provide an accurate measure of this bulk isothermal compressibility, and, as shown below, the compressibilities as they change with weight percent PEG vary with molecular mass. However, literature values of both the fugacities [4,5] and the osmotic pressures [6] of aqueous PEG 2000 to PEG 8000 Da remain essentially constant with weight percent. Dijkstra's equation then projects that the osmotic compressibility should be independent of molecular mass. The cause of this conflict between the predicted and experimental results is addressed below.

2. Experimental[1]

2.1. Preparation of samples

Stock solutions of PEG 2k, 4k, and 8k (Fluka, purum: nominal 4k labeled as 3500–4500 Da; nominal 8k labeled as 7000–9000 Da) were 50% w/v and were allowed to equilibrate for 24 h before dilution. The final PEG solutions were prepared from stock to their final concentrations at least 24 h before scattering experiments. However, SANS showed that the PEG 8k solution diluted from a 50% stock solution to its final, equilibrated, monomeric form took longer—four days—and so 8k samples were formulated at least four days before the 8k data was collected. All solutions containing PEG were kept at ambient temperature in air and, to the greatest extent possible, in the dark.

Stock 4 M $(NH_4)_2SO_4$ (Sigma ultra), stock 1 M HEPES buffer (sodium salt and acid forms from Sigma), and stock 10% w/v sodium azide (Sigma) were used. All final solutions contained 0.1% w/v azide as bacteriostat and 10 mM of the buffer. The pD values of the final solutions were measured to be between 6.95 and 7.25 with a glass electrode, with no correction made for isotope effects: the directly measured pD values were judged to be more accurate due to the simultaneous and roughly parallel changes in the buffer and electrode-surface equilibria with changes in the proportions of *H* and *D*.

MALDI spectra of the PEGs were obtained to determine their distributions of the homologous oligomers. The mass distribution of nominal 2k PEG has a peak at 1.85 kDa and half-width at half maximum (HWHM) of 450 Da; nominal 4k has a peak at 4.3 kDa, and HWHM 460 Da; nominal 8k PEG has its peak at 9.0 kDa and HWHM of 750 Da. The relative widths of the homologous series distributions decrease with increasing nominal mass.

[1] Certain trade names and company products are identified in order to specify adequately the procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products are necessarily the best for the purpose.

If evidence for gas bubble scattering was present, the solutions were degassed under vacuum and rerun. The temperature during the scattering experiments was held at 22 ± 1 °C.

2.2. SANS measurements

SANS measurements were performed on the NG7 30-meter SANS instrument at the NIST Center for Neutron Research in Gaithersburg, MD [7]. The neutron wavelength, λ, was 5–5.5 Å, with a wavelength spread, $\Delta\lambda/\lambda$, of 0.11. Scattered neutrons were detected with a 64 cm × 64 cm two-dimensional position sensitive detector with 128 × 128 pixels. Raw counts were normalized to a common monitor count and corrected for empty cell counts, ambient room background counts, and non-uniform detector response. Data were placed on an absolute scale by normalizing the scattered intensity to the incident beam flux. Finally, the data were radially-averaged to produce scattering intensity curves, $I(q)$, versus q. A sample-to-detector distance of 1.3 m or 1.5 m was used in order to cover the range $0.028\ \text{Å}^{-1} \le q \le 0.34\ \text{Å}^{-1}$. Obtaining correct measures of the bulk compressibility requires accurate corrections for the scattering background and especially for incoherent scattering from protons, which varies with h-PEG concentration. Both of these corrections were made utilizing the procedures of Rubinson et al. [2].

The total SANS signal is linearly proportional to the number density of the scatterers, n, and quadratically both to the scatterers' volumes, V, and to their contrast with the solvent, $(\Delta\rho)$. The contrast $\Delta\rho = \rho_p - \rho_s$; here, ρ_p is the scattering length density of the particles such as macromolecules, and ρ_s the scattering length density of the solvent. These contributions are related to the scattered intensity as shown below.

$$I(q) = n_p V_p^2 (\Delta\rho)^2 P(q) S(q) + B(q) \quad (2)$$

where q is the momentum transfer [$=(2\pi/\lambda)\sin\theta$] where 2θ is the scattering angle, n_p is the number density of scatters (p = particles), V_p is the volume of one particle, $\Delta\rho$ is the contrast, $P(q)$ is the form factor (or shape factor), $S(q)$ is the interparticle structure factor, $B(q)$ is the total background signal from the solvent, buffer, cuvette, and solutes.

$S(q)$ can be found from the scattering data alone with the assumption that the particle structures do not change with concentration and the background is fully corrected [2]. If at low concentrations the particles do not interact intermolecularly, $S(q) \equiv 1$, then

$$S(q)_{\mathrm{interacting}} = \frac{n_{\mathrm{noninteracting}} I(q)_{\mathrm{interacting}}}{n_{\mathrm{interacting}} I(q)_{\mathrm{noninteracting}}} \quad (3)$$

The 1% w/v solutions of each of the PEGs were assumed to be intermolecularly noninteracting since their intermolecular average distance is greater than $2 \times R_g$. Three separate sets of calculations to find $S(q)$ were carried out: one for each specific nominal molecular mass at the different concentrations. Any measure of concentrations n can be used with Equation (3), and molarity, weight percent, volume fraction, and number density are convenient. Number density is calculated as $n_p = (\text{molar concentration} \times 6.022 \times 10^{23})$.

Hayter and Penfold [8] showed that the separation of $P(q)$ and $S(q)$ in Equation (2) strictly holds only for homogeneous monodisperse spheres in solution. However, it has been found to hold for nonspherical solutes that are not strictly monodisperse. We shall assume here that Equations (2) and (3) hold exactly rather than as approximations.

The value $S(0)$, the value of $S(q)$ extrapolated to $q = 0$, for the oligomer solution's scattering is related to the isothermal compressibility of the solution by [3,9]

$$S(0) = n_p k_B T \chi_T$$

with unity being the compressibility of the solvent alone. We will use the terms $S(0)$ and the compressibility relative to the water solvent interchangeably. The values of $S(0)$ were found by short, smooth extensions of the $S(q)$ graph to $q = 0$. We estimate the $S(0)$ values themselves to have relative uncertainties less than 5%, and were essentially insensitive to the method of extrapolation within that range.

2.3. Working with previously published data

When literature data were only available from graphs, the graphs were scanned at high resolution and then digitized using the software Un-Scan-It (Silk Scientific, Orum, UT). All curve fitting was done with TableCurve 2D (Systat, San Jose, CA).

3. Results and discussion

From the small-angle neutron scattering we have determined average intermolecular nearest-neighbor distances for PEGs with nominal molecular weights of 2000, 4000, and 8000 Da for concentrations from 3% to 17% w/v as well as the associated bulk compressibilites. As will be discussed, the average intermolecular distances are easily predictable. However, the compressibilities have no simple quantitative explanation but share some characteristics with aqueous ethanol solutions.

3.1. Intermolecular spacing

The $S(q)$ curves for PEG 4k are shown in Fig. 1. Here, as expected, the maxima of the curves q_{peak} indicate the average intermolecular PEG spacing; the molecules are separated by an average distance of $2\pi/q_{peak}$. For PEG 4k, the measured intermolecular center-to-center separations are listed in Table 1.

The center-to-center distances in column 1 of Table 1 were calculated from the properties of cubic close packed spheres, where each sphere represents the volume available for each PEG molecule. The volume of a unit cell, which contains four spheres, is $(16\sqrt{2})r^3$, so the volume available to each molecule is one-fourth of that amount; this volume is 5.65 r^3. This combined with the number densities of the PEGs, provides the distances $2r$ in column 1, the expected center-to-center distances.

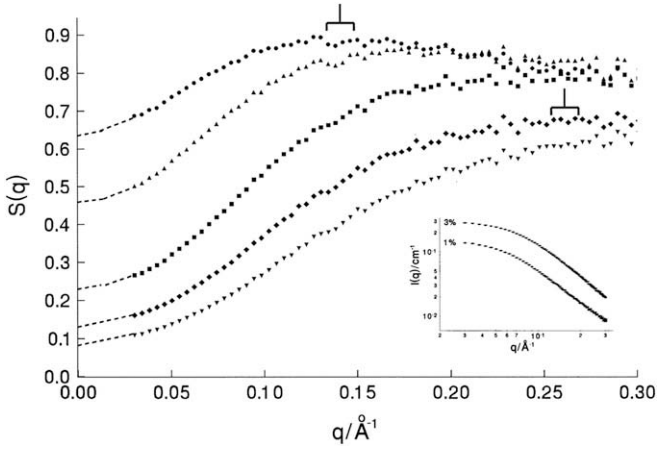

Fig. 1. $S(q)$ curves calculated point by point for PEG 4000 in D_2O. Concentrations increase from top to bottom: 3%, 5%, 9%, 13%, 17%. Equation (3) was used assuming that at 1% w/v the polymers do not interact—that is, the molecular motions are not correlated at 1% w/v. The value of q_{max} is shown for two of the curves along with an estimated error bar along q. The errors in $S(q)$ are smaller than the points in the range below $q \approx 0.15$. Extrapolations to $S(0)$ are shown as dashed lines, and the values of $S(0)$ are estimated to be ±5%. The inset shows data points ln $I(q)$ versus ln q with error bars for 1% and 3% PEG 4000.

Table 1
Center-to-center distances for various concentrations of PEG 4000.

Calculated (Å)	Measured PEG 4k (Å)	Percent w/v concentration
68	45 ± 4	3
57	38 ± 3	5
47	28 ± 2	9
41	24 ± 2	13
38	23 ± 2	17

Experimentally, the average distances as calculated from the peaks of the $S(q)$ curves change with concentration as $n^{-0.41\pm0.03}$ and are about 2/3 of those expected from an evenly spaced set of molecules. The reason for this disagreement is not understood.

The breadth of the curves around their maxima is typical for proteins and for these PEGs and indicate the softness of the repulsive potentials for these solutes compared to those for small molecule liquids, e.g., liquid argon and liquid hydrocarbons, that have been subject to small-angle scattering measurements. Another characteristic of the protein and PEG solution curves is their slow rise toward unity at higher q values.

The origin of the intermolecular repulsion may be simply occasional contact between the PEG oligomers. For example, as shown in Table 2 in the third column, for 17% w/v PEG, twice the measured radius of gyration $(2 \times R_g)$ approximately equals the intermolecular separations for all three molecular masses. $S(q)$ curves should reliable to that level. (On the other hand, the 1% solutions have the average intermolecular separation much larger than $(2 \times R_g)$ as shown in the second column of the table, which supports the noninteracting assignment to this concentration in Equation (3).)

3.2. The solution compressibilities

The measurement of $S(0)$ provides a measurement of the relative compressibilities of the PEG solutions [9] with the relative compressibility of the solvent being unity. These measured values are plotted in Fig. 2.

The solution compressibilities measured by SANS in essence are derived from the spatial correlations between the scattering particles with the particles themselves serving as probes for the compressibility they experience with their k_BT-induced motions. Since the scattering depends on the difference in the scattering length densities of the particles and the solvent, $\Delta\rho = \rho_p - \rho_s$, the polymers act as unhydrated probes of the surrounding bulk solution. The measurement with SANS, then, differs from those using ultrasound or other bulk measurements, where the measurement reflects changes in both the polymer and the water structures. The relative compressibility measurement done here with SANS provides unprecedented sensitivity at this range of concentrations.

It is clear that the isothermal compressibilities differ depending on molecular mass. Below about 6% w/v, the effectiveness for

Table 2
Selected PEG molecular and solution structural properties.

PEG molecular weight (Da)	Calculated intermolecular distance 1% w/v (Å)	Calculated intermolecular distance 17% w/v (Å)	Experimental measure of PEG diameters: $2 \times$ PEG R_g (Å)
2000	77	30	24
4000	98	38	38
8000	123	47	52

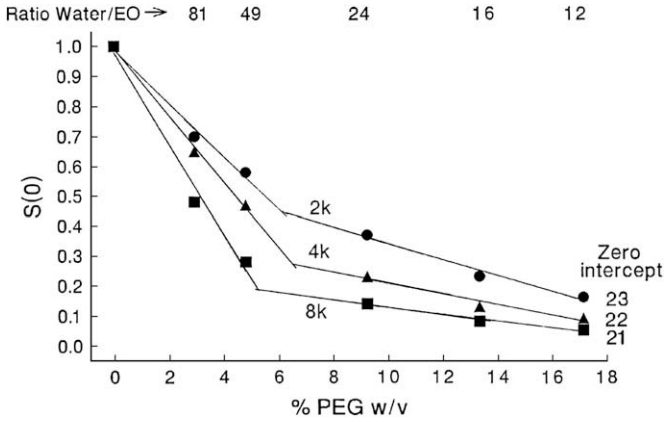

Fig. 2. $S(0)$ versus percent w/v for PEGs in D_2O. The lines are best fitting for the two sets of three points for each molecular weight. The points indicate relative values of the solution's bulk compressibility. Estimated errors are approximately the size of the points.

a given amount of added PEG to change the compressibility increases with molecular mass. However, as seen in Fig. 3, over the mass range 2000–8000 Da and the concentration range investigated, the osmotic pressures of PEG solutions do not depend on molecular mass. In addition, as seen in Fig. 4, the activities of water in these solutions also are independent of molecular mass. As a result, the first term in square brackets in Dijkstra's equation, Equation (1), predicts no dependence on molecular mass, which is contrary to the observed behavior.

To describe the solution changes that are observed, we borrow two phenomenological labels from polymer solids—antiplasticization [10,11] and composite properties. Antiplasticization is a term applied to stiffening by additives; their addition decreases the compressibility compared to the pure material. Qualitatively, the effect of the PEG on the water is to act as an antiplasticizer. This nomenclature avoids an assumed specific structural cause that would be implied by using terminology such as chaotropic/kosmotropic (which usually is applied to the effect of ions on a solvent) or structure breaking/structure making (which assumes experimentally assessable structures for the solvent hydrogen bonding). For polymers, the antiplasticization stiffening occurs when adding small molecules to solid polymers. Apparently the effect results from the decrease in size of the intermolecular void regions [12], which provides a more specific structural description than simply asserting a decrease in the free volume. Since the PEG is significantly larger than the water molecules, however, another molecular-level explanation must be sought.

Toward that end, we note that a linear change with volume fraction in a physical property such as compressibility is one characteristic of a solid composite material [13,14]. The composite material's given mechanical property equals a sum of terms equal to the volume fractions of the components times their individual values of the same mechanical property. This linearity generally holds up to 20–30% volume fraction [13,14]. As a result, we can label the PEG–water system as a composite solution. This type of additivity also is assumed to be valid to calculate partial molal volumes for low concentrations of solutes. However, here we cannot in any rigorous way apportion the changes in compressibility to water alone or to PEG alone.

We have fit the compressibility data for each molecular mass with two line segments joined at a break in slope as seen in Fig. 2. The existence of a break is supported by infrared spectra run on the same PEGs over the same concentration range. The infrared absorption coefficients of many of the vibrational bands were

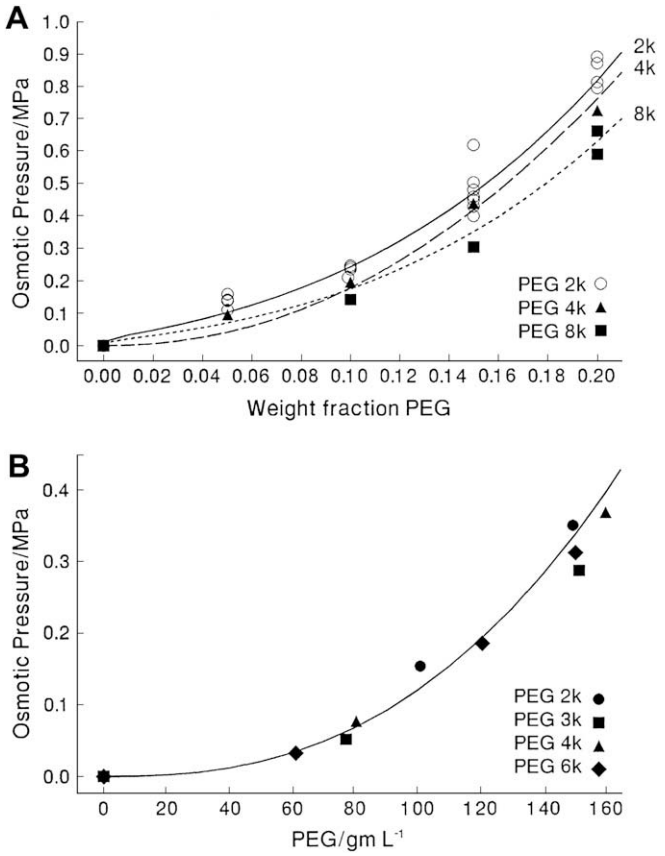

Fig. 3. Plots from two literature sources of the osmotic pressures versus mass concentrations of PEGs with different molecular weights in aqueous solution. The mass concentration ranges approximately to that of Fig. 2. (A) Data from lpsb.nichd.nih.gov/osmotic_stress. The repetitive points, especially for PEG 2k, indicate the uncertainties of the measurements. (B) Data is from Money [4]. The single curve is a best fit power function for the data of all the molecular weights together.

discontinuous near the same concentration as the break in solution compressibility.

The explanation of the observed results follows the general ideas of titration; the added PEG titrates the water. As long as free solvent water molecules are available, the linear decrease in compressibility with mass of PEG immediately follows, since each

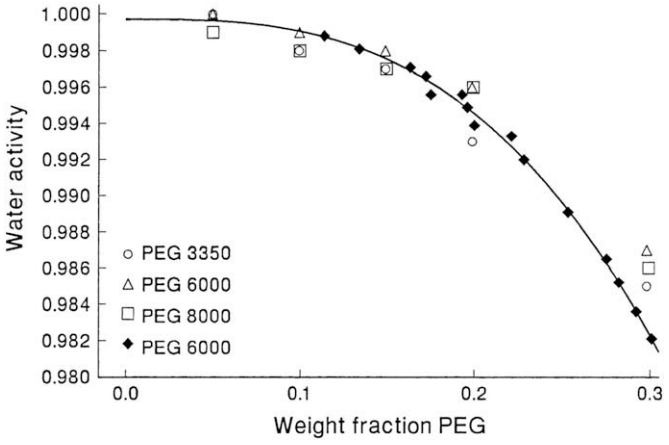

Fig. 4. The activity coefficient of PEG solutions of different molecular weights as functions of weight fraction PEG. Points with open symbols are from Ninni et al. [23] with $T = 298$ K. Points with closed symbols are from Grossmann et al. [6] with $T = 293.15$ K.

PEG molecule independently interacts with all the water it can. This linearity is expected until all the waters are associated with PEG molecules. From the differences in slopes, the effectiveness in associating with water increases with molecular mass, and the use of all the available waters is reached at lower w/v levels with the PEG 8000 than for the smaller ones.

After the break points, where the slopes change, the outer waters must begin to be shared, and the change in compressibility with concentration must be lower since all the water has already been associated. More added PEG can only modify already-associated waters with the difference (slope) being less than when free solvent was available. At some concentration, the PEG chains become so close that the boundaries of the covalent oligomers do not matter; PEGs with different molecular weights form the same extended structure, and the compressibilities of the three different oligomer masses become equal. This occurs near 20%. It necessarily follows that the PEG that initially is most effective at changing the compressibility, PEG 8000, after the break must have the shallowest slope, with PEG 4000 being steeper, and PEG 2000 the steepest in that region.

3.3. Changing compressibilities: comparison with aqueous ethanol solutions

The breaks in the compressibility curves within the range 0.05–0.07 w/v indicate when the PEGs have, in effect, titrated all the free water molecules. From this range of weight fractions, we can infer that each ethylene oxide monomer controls in this range 46–32 water molecules. The apparently equivalent break in the ethanol partial molar volume curve at 6.4%—the minimum of the derivative in Fig. 5—similarly suggests that each ethanol controls about 37 waters—a value in the middle of the PEG–monomer range. This large number of waters per monomer group requires numerous layers of hydration to be affected. Such multilayer associations have been found from neutron scattering of aqueous *t*-butanol solutions where the ordering can be measured even in the third layer in the direction projecting from the alcohol group [15].

An extensive literature exists describing the chemical structures in aqueous ethanol solutions as inferred from a wide variety of measurements. The complexity of the chemistry over the same concentration range as the PEG solutions here is indicated by Frank's measurement of ethanol's partial molar volume [16,17] as graphed in Fig. 5. The derivative indicates clearly a change at about 2% (w/w), with an extreme at about 6.4%. Further maxima in the derivative appear at 11% and 20% and a minimum at 16%. Numerous interpretations have occurred over the years, but many seem to have been guesses made without understanding that the ethanol molecules form clusters as shown by Nishi et al. [18]. They established that at 35 °C some dimerization of ethanol appears even in 28 mM solution (0.0013% w/w, 0.0005 mol fraction), and hydrated ethanol trimers, tetramers, and pentamers are present by 0.55 M (2.5% w/w, 0.01 mol fraction). Matsumoto et al. [19] found from X-ray scattering that the clusters formed by ethanol molecules have adjacent ethyl groups aligned in parallel, and the –OH groups forming hydrogen bonds with water all lie on one side of the hydrophobic plane. In contrast, the PEGs' chains with their etheric oxygens do not have the flexibility to match the structures of ethanol clusters, but we expect that the same tendency exists toward clustering of the alkane groups.

Ludwig [20] notes from NMR relaxation studies that addition of small amounts of alcohols to water makes the water "more solid-like," and the hydrophobic interactions are such that an alcohol should be considered a "soluble hydrocarbon" rather than an "alkylated water." His data, nevertheless, did not exhibit any breaks in the linear changes with alcohol concentration over the range from about 6 to 22 weight percent ethanol. As can be seen from the smooth curves of Figs. 3 and 4, unlike the osmotic pressure and water activity measurements the compressibilities of the PEG solutions are sensitive to the changing PEG solutions' structures.

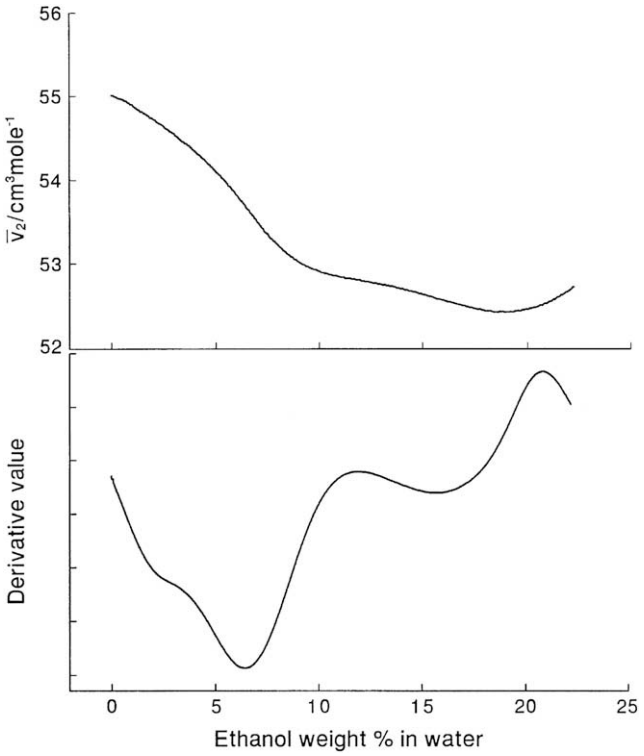

Fig. 5. Top: Partial molar volume of ethanol in water versus weight fraction. Bottom: The derivative of the top curve. Data from Franks [16,17].

3.4. Comparison of compressibilities of neat PEG and in aqueous solution

Neat PEG 2000, a waxy solid, exhibits an isothermal compressibility typical for many organic substances [21]: 0.39 GPa^{-1}. Water's compressibility is in that range as well [22]: at 20 °C it is 0.46 GPa^{-1}.

For the PEG–water system, we should not assume that the average water density remains constant at the pure solvent's value; the PEGs have a significant effect on the structure of the water, and vice versa, as seen in the compressibility changes of the solution. Franks [17] said this about the ethanol–water solution: "…although alcohols are more compressible than water, small additions of an alcohol to water cause a decrease in compressibility, just as if some compression-resistant structure were being formed or fortified." And, "Simple H-bond making and breaking is difficult to reconcile with the other complex properties of this system." The same must be said of the PEGs.

Although a clear separation between the changes in PEG and water can neither be assumed nor supported quantitatively, we can obtain approximations by assuming the changes in the PEGs are much greater than those of the water. In the limit, if the entire compressibility change were due to the PEG alone, then the slopes of the compressibility graph at the low concentrations (below the break) show the ratios of compressibilities of water to PEG are: water/PEG 2000 = 8; water/PEG 4000 = 10; and water/PEG 8000 = 16 on a volume basis. From the expected behavior of a composite solvent—that is, changes of the measured compressibility with the volume fractions of the two materials—we can say

that the presence of sufficient water decreases the compressibilities of the solute PEGs by an order of magnitude.

4. Conclusions

The changes in aqueous solution compressibilities from adding PEGs in the molecular mass range 2000–8000 Da show their influence on the structure of water around them through many layers of hydration. Concurrently, the water changes the structures of the PEGs, which causes them to be an order of magnitude less compressible than when dry. These results are inferred from classical chemical arguments using the stoichiometry, masses, volumes, and compressibilities of the neat materials compared to their solutions. The antiplasticization of the aqueous solvent by the PEGs and the linear change in the solutions' compressibilites with PEG are conveniently encompassed by the concept of a composite solution. This composite effect should be ubiquitous and have significant parallels in crowded solutions such as cytoplasm and gels.

However, when applying a quantitative thermochemical relationship such as Equation (1), it fails to capture the observed changes. Dijkstra [3] notes that Equation (1) holds when pairwise additivity of the primary interaction potentials applies. Pairwise additivity means that the interaction potential function can be written in terms of a sum of all the positions and orientations of pairs of molecules alone. The hypothesis of pairwise additivity must be tested, and in this relatively simple polymer system of PEG in an aqueous buffered salt solution, the hypothesis fails. This failure is, indeed, expected in substances with strongly orientation-dependent interaction energies such as those occur with hydrogen bonding and hydrophobic interactions around solutes in water. Certainly, interactions between a structured solvent such as water with an ethylene oxide site changes the binding among other waters in proximity. As a result, although the pairwise additivity of primary interaction potentials is a reasonable assumption for simple fluids and fluid mixtures, it is not valid for PEG in aqueous buffered salt solution.

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