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Equation of state of PEG/PEO in good solvent. Comparison between a one-parameter EOS and experiments.

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ABSTRACT

We investigate, through osmotic pressure measurements, the validity of the singleparameter equation of state (EOS) for solutions of polyethylene glycols in water, by Cohen et al.^{1,2} We show that it is physically meaningful and that a fairly good correspondence between the osmotic pressures for PEG35 in large range of concentrations is obtained. We also take the chain length dependence into account in our analysis, as suggested by Cohen et al. By recalculating the experimental pressures in the paper by Jönsson et al.³ applying the new calibration curve, which is based on the experimental results obtained in this study and the EOS obtained by Cohen et al., there is almost a perfect correspondence between the simulations and the experiments. These results have implications for correctly probing macromolecular interactions in wide range of systems when applying the osmotic stress method.

1. Introduction

Polyethylene glycol (PEG), also known as polyoxyethylene oxide (PEO), is a polyether compound and a commonly used polymer, with many applications from manufacturing to medicine. It exists in a variety of molecular weights, $O(10^2) - O(10^7)$ g/mole, and it is considered to have complex interactions with water. Furthermore, the properties of PEG are often found to depend critically on the molecular weight and less on the concentration.⁴⁻⁶ PEG is a commonly used molecule in the osmotic stress method to quantify macromolecular interactions. *The osmotic stress method of probing the thermodynamics and the intermolecular interactions of macromolecular solutions relies heavily on the PEG osmotic equation of state (EOS).* Usually PEG with a molecular weight of 35 kDa is used for this purpose.

In a multicomponent system that exchanges "solvent" molecules with the rest of the world, osmotic pressure is the force that acts on the concentration of these solvent molecules.⁷⁻¹⁰ At equilibrium, osmotic pressure is usually applied through exchange of solvent molecules across a semipermeable membrane or a vapor phase that prevent loss of solute molecules. When a semipermeable membrane or any kind of liquid state device is used, the process through which these molecules are exchanged is called osmosis. The process of osmosis is used in an astounding number of applications, including most processes that are coupled to concentration variables such as drying of paints and coatings,¹¹ dehydration and rehydration of proteins,¹² and dried foods,¹³ concentration of nanoparticles,¹⁴⁻¹⁶ soft colloids,¹⁷⁻¹⁹ and pastes,^{20,21} desalination of sea water, and concentration of ceramic pastes either by hot pressing or else though casting processes.²²⁻²⁵

In a two-component system, the variation of osmotic pressure with volume fraction of solute over the complete range of volume fractions (from zero to maximum volume fraction of the solute) is the equation of state of the system (EOS), i.e. it determines, in equilibrium conditions, how much solvent will mix with any given solute through a membrane exchange or through a vapor-phase exchange.^{3,26} In aqueous systems, which are by far the most important to us, the EOS of a few polymer and protein solutions as well as those of some nanometric colloidal dispersions have been determined over very wide ranges of pressures and volume fractions.^{3,11,12,18,24,27,28} In turn, the knowledge of these EOS has been used to validate or invalidate models that describe, at high concentrations, the interactions between solute macromolecules, between dissolved proteins, between dispersed particles, and between emulsion droplets. However, in order

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to explore such wide ranges of volume fractions (0 to 0.6), it has been necessary to use membrane and vapor-phase exchange with "standard" polymer solutions and saturated salt solutions. Thus the whole edifice of solution thermodynamics rests on the confidence that we may have in the EOS of these "standard" polymer solutions. However, centrifugation experiments are an exception to this rule.^{19,26,29}

At present we do not "know" the EOS of any polymer solution with perfect precision. From a theoretical point of view, this is because we do not have an exact theory of pure liquids, binary molecular solutions, or macromolecular solutions. However, in special cases, we do have approximate EOS that does a very good job. These approximate EOS are either virial EOS or non-virial EOS. The virial equations start with the ideal pressure of non-interacting solutes and then add correction terms for two-body and three-body interactions between solute molecules. A well-known example is the Carnaham-Starling EOS, which has an amazing range of accuracy in the case where the solute molecules or dispersed particles behave as hard spheres with no interactions beyond contact.⁷ In the early years of colloid science, some EOS of colloidal systems were established using PEG solutions.^{9,14,15,17,28}

For solutions of polymers in a good solvent, virial EOS are inadequate, because the dissolved macromolecules expand throughout the solvent and therefore have excluded volume interactions that are transmitted through the chain over very large distances from any given chain segment.³⁰ In the semidilute range of concentrations, where scaling laws may apply, Jannink and des Cloizeaux have proposed a scaling form of the EOS, where the osmotic pressure is expressed as combination of power laws of the reduced concentration C/C^* , where C* is defined as the polymer overlap concentration.³¹

Cohen et al.^{1,32} have assessed the accuracy of the des Cloizeaux scaling expression for the EOS in the case of aqueous solutions of PEG ($M_w = 300-20\ 000\ Da$), and found good agreement at concentrations that are in the semi-dilute range, provided that the prefactor α of the scaling law was adjusted. Initially, they claimed that a single value of α was adequate to obtain this agreement for all PEG solutions, and therefore that they had obtained a "single parameter EOS". At lower concentrations where the polymer solution was dilute rather than semidilute, they found pressures that were higher than the scaling law, due to the fact that the macromolecules have more degrees of freedom when they do not overlap. Then they made the statement that at all concentrations the osmotic pressure of a polymer solution could be approximated by the sum of the van't Hoff ideal

pressure of the macromolecules and the scaling pressure calculated at the actual concentration through the Jannink- des Cloizeaux expression with the adequate prefactor.

Thus the validity and accuracy of the EOS that have been determined for all polymer and protein solutions as well as for most colloidal dispersions and emulsions depend on whether we consider that we have obtained satisfactory answers to the following questions:

- 1) What are the theoretical validity and the range of application of the theoretical approximation by Cohen?
- 2) What is the accuracy of currently available measurements of osmotic pressures of PEG solutions?
- 3) What are the consequences of the validation of the theoretical equation of state for the EOS of colloidal systems that have been established using different EOS for PEG in water?

2. Experimental Section

2.1 Materials

Samples of polyethylene glycol of molecular weight (MW) 35 000 g/mol (PEG35) from different sources (Aldrich, Fluka (Germany), Fluka (Switzerland), Sigma) were used whereas polyethylene glycol of MW 20 000 g/mol (PEG20) was purchased from Fluka (Germany). The two different PEG35 were dialyzed against milliQ water (Millipore gradient A10) in dialysis bags with molecular weight cutoffs (MWCO) of 3 500 g/mol and 12 000-14 000 g/mol respectively (spectra/ pore 3 and spectra/pore 4 by Spectrum Laboratories, Inc.). Approximately 10 mL of a 11.7 (w/w) % solution of PEG35 in water were initially introduced in a bag of twice capacity. The sample was then dialyzed for two weeks in five different baths of 400 mL each. After the dialysis, the content of the bag was freeze dried to recover the polymer powder. The yield for the dialysis was 90 % with the 3 500 g/mol MWCO bag, and 60 % for the dialysis with the 12 000-14 000 g/mol MWCO bag.

2.2 Methods

Samples for osmotic pressure measurements were prepared by weighing a certain amount of solid PEG and dissolving the powder in a weighed amount of milliQ water. For the osmotic pressure measurements, a membrane osmometer (Osmomat 090, Gonotec, Berlin, Germany) was used with cellulose acetate semipermeable membranes, MWCO equal 10 000 g/mol. Measurements with the osmometer were performed according to the instruction manual of the apparatus. Briefly, the instrument consists of a sample chamber

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and a reference cell including a pressure transducer separated by a semipermeable membrane. Due to the osmotic pressure difference between solvent and solution, a vacuum, which is related to the concentration of the osmotic actively solute particles, is generated in the lower cell half. The measurement involved calibration of the pressure transducer and stabilization of baselines. The measurement chamber was filled with polymer solution three times while continuously monitoring the osmotic pressure. In particular, for a first equilibration of the membrane, the apparatus was rinsed several times, approximately twelve hours of equilibration in total with milliQ water. The samples were prepared by gravimetry without any dilution step. For a given sample in a given measuring session, the pressures were determined in series of solutions of increasing concentration. Several measurements were made for each sample to ensure that there were no dilution effects. For each measurement, the pressure was followed during several minutes and the measurement never exceeded six minutes. The rates at which the pressure evolve range from less than -1 Pa/min to -20 Pa/min in the case of the most concentrated samples. Due to the flexible conformation of the polymer, a certain amount of leakage is unavoidable due to reptation through the pores of the membrane. Generally, the readings of the pressures were done three to four minutes after the end of the injection, which gives a reasonable equilibration time while keeping the leakage to a minimum. All experiments were performed in room temperature i.e. 25° C.

3. One-parameter scaling EOS

Throughout this paper the results will be compared with the one-parameter scaling EOS for osmotic pressures of polymer in good solvent systems suggested by Cohen et al.¹ In brief, the derived equation is a non-virial linear combination of a low-concentration van't Hoff (vH)³³ term and a higher-concentration des Cloizeaux (dCl)³⁴ term. A single parameter α locates the crossover from dilute vH to semidilute dC behavior. The value of α was determined by an empirical fit to experimental data for PEGs of molecular weights ranging from 300 to 20 000 Daltons.²⁸ A detailed derivation of the proposed scaling EOS, is found in ref.¹

$$\Pi N^{9/5} = \frac{RT}{M_m V} \left[\left(\frac{C}{C_N^*} \right) + \alpha \left(\frac{C}{C_N^*} \right)^{9/4} \right]$$

(1)

In eq. 1, Π is the osmotic pressure, *N* is the number of monomers per polymer chain, *R* is the universal gas constant, *T* is the temperature, \overline{V} is the polymer partial specific volume, *C* is the polymer mass concentration. Furthermore, *C** is a characteristic *N*-dependent polymer concentration associated with the crossover between dilute and semidilute regime defined as: $C^* \equiv N^{-4/5} / \overline{V}_{,}$ and corresponds to a semi-quantitatively defined polymer overlap concentration.^{30,35} α is defined as the crossover index, and the crossover concentration is defined as the concentration where $\Pi_{vH} = \Pi_{dCl}$ i.e. at $C/C_N^* = \alpha^{-4/5}$. When plotting $\Pi N^{9/5}$ versus C/C^* all experimental data for different PEG molecular weights (in the range 300 g/mole to 20 000 g/mole) collapse on a master curve.^{28,36} Fitting such curve with eq. 1 gave $\alpha = 0.49$ for $\overline{V} = 0.825$ mL/g and $M_m = 44$ g/mole.

$$\alpha(N) = \alpha^* \lfloor 1 + bN^{-p} \rfloor$$

(2)

with $\alpha^{\mathbb{Z}} = 0.43 \pm 0.02$, $b=5.3 \pm 1.4$, and $p = 0.84 \pm 1.5$. Applying eq. 2 to PEG20 and PEG35, which are of interest for this study, an α alpha of 0.44 is achieved. The increase of α a \cdot low N mimics the behaviour in the semidilute regime due the increased ability of entanglement.

Since the aim is to validate the theoretical EOS, we have throughout this study used the semi-quantitative overlap concentration, C*, defined by Cohen et al., which becomes 9.04 mg/ml and 5.8 mg/ml, for PEG20 and PEG35 respectively. Comparison by estimating the overlap concentration as the volume one polymer molecule occupies in a sphere, where the radius is set equal to the polymer radius of gyration gives 23.9 mg/ml and 15.9 mg/ml for PEG20 and PEG35 respectively. In the latter the relation for radius of gyration as

published by Devanand et al.³⁸ were applied. There is a difference by an approximately a factor of three between the semi-quantitative overlap concentration and a commonly used overlap concentration. Hence the parabolic appearance as shown in the experimental EOS is probably an effect of crossing the actual overlap-concentration.

4. Results and Discussion

4.1 Osmotic pressure measurements

The osmotic pressure values for three different sets of samples of PEG35's using the membrane osmometer are presented in Figure 1. The pressure values span nearly three decades in pressure, from 10 Pa to 10⁴ Pa. Set one (S1) corresponds to dialyzed PEG35, set two (S2) to undialysed PEG35 obtained from the same source as S1, whereas set three (S3) are an old source recovered from stocks used in earlier experiments.^{17,39} On a log-log scale, all data coincide on one master curve, and there seems to be no specific effects with respect to ageing.

Both the van't Hoff law and the EOS, presented in the theoretical section, assume that the solution contains a single population made of identical macromolecules i.e. monodisperse solution. Indeed if the solution contained another population of macromolecules with a lower degree of polymerization, this population could dominate the pressure because, at a given mass concentration, the small macromolecules can be much more numerous.¹⁴ Comparison with PEG solutions equilibrated against pure water across dialysis membranes (MWCO = 3.5 kD) did not give any pressure changes; which indicates a single narrow population of PEG macromolecules. This is in line with the study by Kawaguchi et al.⁶ who showed that for PEO20 in water at 25° C, no aggregates exists in water. Hence, from the perspective of accuracy of the osmotic pressure measurements, purification by dialysis is not necessary.



Figure 1. Net osmotic pressure in Pascal as a function of concentration in mg/ml for three different sets of PEG35 where set S1 corresponds to dialyzed PEG35 (filled black circles), set S2 corresponds to undialysed PEG35 (open red squares), and set S3, an old source of PEG35 (open blue diamonds).

Focusing on PEG35 in Figure 2, a linear fit to the different data sets gives the following molecular weight and second virial coefficients (A_2) respectively: S1 (31 400 g/mole, 2.14*10⁻³ mole*cm³/g²), S2 (30 700 g/mole, 2.13*10⁻³ mole*cm³/g²), and S3 (29 900 g/mole, $1.72*10^{-3}$ mole*cm³/g²). The linear regression coefficients are 0.9859, 0.9798, 0.998 for S1, S2, and S3 respectively. Notice that what is expected to be an almost perfect fit on the logarithmic scale, depicts rather large deviances on the linear scale. The reason to why the pressures curves have a slight parabolic appearance is probably due to the passing the polymer overlap concentration.

The second virial coefficients for PEG35, A_2 , span the region $1.72-2.34*10^{-3}$ mole*cm³/g². Devand and Selser³⁸ concluded that the second virial coefficient depends on the molecular mass of the polymer according to: $A_2 = 1.84*10^{-2} M_w^{-0.20\pm0.06}$, which gives a good correspondence with the values we have obtained in this study. Furthermore, it shows that the second virial coefficient decreases as the molecular mass increases. On the other hand, Hasse et al.⁴⁰ came to the conclusion that A_2 does not depend on the molecular mass if the third virial coefficient is taken into consideration, whereas Schäfer⁴¹ claims that second virial coefficients are effective parameters that depend on the method used in data analysis and that they are not true virial coefficients.



Figure 2. Reduced osmotic pressure as a function of concentration for set S1 dialyzed PEG35 (filled black circles), set S2 undialysed PEG35 (open red squares), set S3, an old source of PEG35 (open blue diamonds), and set S4 undialysed PEG20. Shown in the Figure is also a linear fit (see eq. in insert) where the intercept gives the inverse number average molecular weight and the slope corresponds to osmotic second virial coefficient, B_2 .

In 2002, Dormidontova⁴² published a thorough theoretical study about PEO in aqueous solution applying a mean-field approach that included the effect of the competition of PEO and water as proton acceptors in hydrogen bond formation, with focus on concentration and temperature effects. Regarding A_2 , the theoretical prediction is that the part of the coefficient that reflects specific interactions between PEO and water decreases exponentially with an increase in temperature (Figure 9). This is also confirmed experimentally, ^{43,44} and it is probably due to the decrease in the degree in association between PEO and water i.e. disruption of hydrogen bonds. In the temperature range 20-30° C, the theoretical predictions by Dormidontova⁴² for A_2 show a good agreement for PEO16 by applying the lowest critical angle dependence of PEO-water association as well as the weakest temperature dependence. For the largest PEO as they used for comparison, PEO32.8, larger discrepancies are visible between theory and experiment. The trend is that the shorter polymers gives rise to a better agreement with theory as well as higher temperatures. Hence, it seems to be a decrease in degree of association between PEO and water as the polymer length is increasing i.e. A₂ seems to become less sensitive to hydrogen bonding between PEO-water.

Let us focus on the osmotic second virial coefficient, B_2 , and compare with other results in the literature. B_2 has the unit of volume and can be considered as a characteristic volume associated with a polymer chain in a good solvent. In good solvents, the polymer molecule is often regarded as a random coil, and two coils tend to repel each other. The osmotic second virial coefficient can be estimated approximating the potential of mean force as interactions between two hard spheres with a radius comparable to their radius of gyration, R_g , i.e. $B_2 \propto R_g^3$. Since $R_g \propto N^v \propto M^v$ and $v \approx 0.6$ in a good solvent, the following scaling relation is obtained: $B_2 \propto M^{3v}$, which give a A_2 dependence as $A_2 \propto M^{3v-2}$. In a good solvent, it is expected that the osmotic second virial coefficient follow $B_2 \propto M^{1.8}$. Figure 3 shows ln B_2 against ln M_n , for our data as well as data by Wang et al.⁴⁵



Figure 3. The second virial coefficient, B_2 , as a function of the number molecular mass, M_n , on In-In scale. Data points by Wang et al. are represented as open circles, and data from this study by filled circles.

By representing the data in this set-up, the data by Wang et al. gives a slope of 1.86, whereas the slope obtained from our two values (only) is 1.77. By taking all the data into consideration i.e. ours as well as the one by Wang et al., gives a slope of 1.60. Hence, reasonable good agreement with expected theoretical predictions is achieved, which is a further confirmation of the accuracy of the experiments. The experimental results by us and Wang et al. are also in agreement with the experimental A_2 by Strazielle⁴³ as well as with Venohr et al.⁴⁴ applying static and dynamic light scattering.

Another possibility to determine B_2 is to do a virial expansion and plot the reduced pressure, $P_{\rm b}$, as a function of the average monomer density, $\rho_{\rm P}$, where the former is defined as $P_p \equiv (P_{exp} / k_p T) b^3$ and the latter as $\rho_p = (C * b^3 * N_p) / M_p$. Here all terms beyond the third order are neglected. Notice that these values are normalized with the monomermonomer bond length, set to b = 4 Å, and the M_n to 35 000 g/mole and 20 000 g/mole respectively. If accurately performed experiments the intercept shall be one and the slope corresponds to $B_{3} * b^{3}$. This is represented in Figure 4 for the two model polymers used in this study, and as shown for PEG20, an intercept of 1.0 is achieved, whereas for PEG35-S2, it becomes 1.15. The obtained osmotic virial coefficients become 1.59*10⁻¹⁸ cm³ and 4.34*10⁻¹⁸ cm³, for PEG20 and PEG35 respectively. If the lowest concentration for PEG35 is neglected due to uncertainties in measurements (resolution of the osmometer), the intercept becomes 1.05, and the linear regression coefficient 0.99024. Hence a fairly good agreement is obtained for PEG35 as well. Comparison with the values given in Table I, where the relation between A_2 and B_2 was applied to achieve the osmotic second virial coefficients, shows that the two methods gives the same value of B_2 , whereas for PEG35 a slightly larger B_2 is obtained in this approach.

System	M _{n, osm}	A_2	B ₂	Regression
Denotation	[g/mole]	[mole*cm ³ /g ²]	[cm ³]	coefficient
S1	31 400	2.14*10 ⁻³	3.5*10 ⁻¹⁸	0.9859
S2	30 700	2.13*10 ⁻³	3.3*10 ⁻¹⁸	0.9798
S3	29 900	1.72*10 ⁻³	2.6*10 ⁻¹⁸	0.998
S4	20 400	2.34*10 ⁻³	$1.6^{10^{-18}}$	0.9922

Table I. The second virial coefficient B_2 , estimated M_n , as well as the regression coefficient for set S1 dialyzed PEG35, set S2 undialysed PEG35, set S3, an old source of PEG35, and set S4 undialysed PEG20.

More remarkable is that the molecular weights diverge by 4000-5000 gram/moles in comparison with the expected value given from the manufacturer. The question is if this is a general trend when obtaining molecular weights from osmometry or if it is a discrepancy for PEG35? The linear fit of PEG20 gives a number molecular weight of 20 400 g/mole and a second virial coefficient of $2.34*10^{-3}$ mole*cm³/g², which is perfectly in line with expected values. The linear regression coefficient is 0.9922. The size dependence regarding polymer length is also captured accurately.⁴⁰

In the following part of the paper, focus will be the two data sets, S2 (undialysed PEG35) and S4 (undialysed PEG20) at room temperature i.e. 25° C.



Figure 4. The reduced pressure P_b as a function of the average monomer density, ρ_p . Notice that the values are normalized against the bond length (4 Å) and thereby no unit.

4.2 Comparison with theoretical EOS

In this section, experimentally obtained pressures will be compared with the theoretical osmotic pressures obtained from the EOS. Comparisons between second virial coefficients and molecular weights are not reasonable since there is concentration dependence in B_2 in the latter, see $d(\Pi N^{9/5})/dC$, which contradicts what the osmotic virial equation describes i.e. B_2 is not constant for low concentrations.

Figure 5 shows the ratio between the des Cloizeaux/ dilute van't Hoff terms in the EOS as a function of the concentration, where dCl/V_H < one implies that the ideal regime is dominating. For concentrations above 10 mg/ml for PEG35 and 17 mg/ml for PEG20), contributions from the semidilute regime i.e. des Cloizeaux term are dominating. When the ratio between the des Cloizeaux/ dilute van't Hoff is represented as a function of reduced concentration (C/C*), the data for PEG20 and PEG35 superimpose on one curve for $\alpha = 0.44$, and $\alpha = 0.49$ respectively, and for $\alpha = 0.44$ it occurs at C/C* \approx 2 and for $\alpha = 0.49$ at C/C* ≈ 1.75 (data not shown).



Figure 5. The ratio between the semi-dilute des Cloizeaux/dilute van't Hoff term obtained from the EOS by Cohen et al., for a = 0.44 and 0.49 as a function of concentration in mg/ml. The molecular mass of a monomer was estimated to 44 g/mole, the number of monomers 455 for PEG20 and 796 for PEG35, and the used polymer partial specific volume was 0.825 ml/g.

Figure 6a shows the net osmotic pressure as a function of concentration for S4, and the EOS by Cohen et al., applying two different α i.e $\alpha = 0.44$ (length dependence considered) and $\alpha = 0.49$. First of all, the experimental data and the EOS agree reasonably well, and it is clearly shown that there is a length-dependence in α as predicted by Cohen et al. Secondly, it is a better correspondence for concentrations less than ≈ 10 mg/ml i.e. in the excluded volume regime c.f. polymer overlap concentration of PEG20 is 9.06 mg/ml.

The crossover between the two regimes is of course dependent on α , and for PEG20, it occurs at \approx 17 mg/ml for α = 0.44, and at \approx 15 mg/ml for α = 0.49. When the ideal contribution is dominating, the agreement between the scaling theory and the experiments is increasing. The insert shows a comparison between the experiments and the non-scaled van't Hoff ideal pressure (dotted black line). As clearly depicted by the linear behaviour in the dilute regime, the ideal regime is reached with PEG20, which confirms the accuracy of using a linear fit to obtain molecular weight.



Figure 6. Net osmotic pressure in Pascal as a function of concentration in mg/ml for experimentally obtained pressures and predicted pressures by EOS by Cohen et al. using $\mathbb{P} = 0.44^2$ and 0.49,¹ for (a) PEG20 and (b) PEG35, undialysed. The insert shows a magnification of the lower concentration regime, where the dotted black line corresponds to the ideal pressure.

The corresponding analysis for S2 (PEG35) is shown in Figure 6b, applying the same $\alpha \mathbb{PP}$. Similar trends are captured regarding the agreement between the EOS and experimental data with respect to the different regimes in the EOS. The crossover occurs at concentrations of approximately 10 mg/ml, \approx two times the overlap concentration, and below these concentrations, the precision of the experiments is excellent with exception for the lowest concentration (less than 1 mg/ml). The inset in Figure 6b shows that it is not possible to reach the ideal regime for PEG35 (ideal pressure dotted black line), therefore a greater value of the intercept is predicted, which decreases the expected molecular weight.

In Figure 6 it is shown that independent of the molecular weight of the polymers, the scaling EOS overestimates the osmotic pressure for concentrations larger than 15-20 mg/ml i.e C \approx 3-4C^{*}, which corresponds to a polymer weight fraction of 0.15-0.20.

4.3 Comparison with different sources of PEG20 and PEG35 data

PEG20

Figure 7 shows the experimental data obtained in this study (red filled circles), by the ones by Rand²⁸ (blue filled circles), in comparison with the theoretical EOS (black full line),

where in the latter, the length dependence was taken into consideration (α =0.44). Notice that the data by Rand served as a basis for developing the theoretical EOS. In Figure 7a the net osmotic pressure is shown as a function of reduced concentration on log-log scale, and as visible, the experimental data from this study and by Rand are complementary, and explore different regimes. Further, it is a good correspondence between the experimentally obtained pressures and the theoretical predication.



Figure 7. Comparison of (a) the net osmotic pressure by us and Rand²⁸ as a function of the ratio PEG concentration/overlap concentration for PEG20 on log-log scale, (b) the net osmotic pressure as a function of the ratio PEG concentration/overlap concentration for PEG20 on lin-lin scale, as well as (c) the experimental pressures normalized by the EOS by Cohen et al.¹ The filled red circles are experiments obtained by the authors in this study, whereas the blue filled circles are experimental pressures measured by Rand et al.²⁸ The black line corresponds to an exact agreement with the EOS by Cohen et al.¹

When visualized on a lin-lin scale, there iare some deviations, and in the more concentrated regime the experimental pressures are higher than the theoretical ones. This is the same trend as was mentioned by Schäfer.⁴¹ The question is then – how large

are the differences? In Figure 7C, the ratio, $\Pi_{net,exp}/\Pi_{EOS}$, is given as a function of reduced concentration. In the lowest concentration regime i.e. C < 3C*, the difference is less than 15%, this also holds for the intermediate and most concentrated regime, with some exceptions, where it reaches discrepancies of 35%.

PEG35

Comparison between our experiments, scaling theory, and other experimental data reported in the literature^{17,39,40,46} for PEG35 shows that the experimental data obtained by us, Hasse et al., and Grossman et al. agree very well with Cohen et al. on log-log scale, see Figure 8a. There are some deviances in the most dilute regime because at low polymer concentrations the activity of water in the polymer solution hardly differs from that of pure water. Nevertheless, the membrane osmometer estimates accurate values for C > 0.1 wt% (0.001 g/cm³), which should be compared with other techniques such as vapour-pressure osmometry, and isopiestic investigations, where the threshold is approximately at 0.05 g/cm³.

Then there are two datasets, one by Persello et al.³⁹ and one logarithmic model fit by Bouchoux et al.¹⁷ that show pressures significantly lower than those predicted by the EOS. The model expression is obtained from experimental data in the range 0.3-500 kPa applying both membrane osmometry (C < 20 wt%) and osmotic stress measurements. One plausible explanation to the deviance could be that these lower pressures are caused by permeation of the PEG macromolecules through the pores of the osmometer membranes and the dialysis bags (cut-off 12-14 kDa). Indeed, it has been shown that the macromolecules from semi-dilute solutions can crawl through narrow pores when the screening length or mesh size of the semidilute solution becomes equal to or smaller than the pore diameter. This permeation may be mistaken for a slow dynamics of the osmometer instrument, and in that case, attempts to wait for "equilibrium" may just deplete the concentration of the PEG solution to be measured. Another possible explanation could be hydration of the polymers, which overestimates the weight of the sample. For example in the study by Kawaguchi et al.⁶ it was shown that PEO has a relatively large hydrodynamic diameter of 9 Å due to the hydration of the PEG chain. Since osmotic pressure is a colligative property i.e. it depends on the number of molecules in the system, the molecular weight as well as the distribution of molecular weight are of importance. For example the PEG35 used by Persello et al. was claimed to have a molecular weight of 39 kDa from the manufacturer whereas Hasse et al. later showed that it was \approx 35 kDa. For example, at 5 mg/ml (C \approx C*) the ideal contribution to the osmotic pressure is 354 Pa for PEG35, whereas the corresponding value is 317 Pa at ambient

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conditions i.e. a decrease in pressure by 10%. Hence a discrepancy by 10% is not unrealistic, but it cannot explain a factor of two (cf. Cohen et al./Persello et al./Bouchoux et al.).

In Figure 8 b the data are represented on a lin-lin scale, and here, one clearly see that there are (rather large!) discrepancies between the theory and experiments, especially at high concentration i.e. $C > 35C^*$.



Figure 8. Comparison of (a) the net osmotic pressure as a function of the ratio PEG concentration/overlap concentration for PEG20 on log-log scale, (b) the net osmotic pressure as a function of the ratio PEG concentration/overlap concentration for PEG20 on lin-lin scale obtained from our measurements, as well as (c) the experimental obtained pressures normalized by the EOS by Cohen et al.¹ The filled red circles are experiments obtained by the authors in this study, the blue filled circles are experimental pressures measured by Hasse et al,⁴⁰ open black circles by Grossman et al,⁴⁶ dotted red line data by Bouchoux et al,¹⁷ and the dotted black line by Persello et al.³⁹ The black line corresponds to an exact agreement with the EOS by Cohen et al.¹

One plausible explanation might be that the scaling relation cannot capture the entanglement or the excluded volume effects correctly. Similar results were obtained by Schäfer.⁴¹ Figure 8C shows that there is a maximum deviance in the dilute regime of 18% whereas for higher concentrations the deviance is almost linearly increasing, and reaches a maximum of 85%, for C \approx 55C*. On the other hand, the agreement regarding the data for Bouchoux and Persello is increasing when the concentration is increasing and there is a crossover between theory by Cohen et al. and the model expression by Bouchoux et al. at C \approx 50C*. Within the studied concentration regime, the data by Persello et al. always underestimate the pressure in comparison with the theoretical EOS.

4.4 Validity of the scaling function

The calculation of osmotic pressure according to Cohen et al. is based on the choice of an interpolation formula that spans both the dilute and semidilute regimes. Figure 9 shows the crossover from dilute to semidilute solutions for PEG40 (to $C \approx 20C^*$). This graph presents the values of the characteristic length of the PEG40 aqueous solutions at 20 °C, according to polymer concentration *C*.



Figure 9. Crossover from dilute to semidilute solutions for PEG 40. Characteristic length of the PEG40 aqueous solutions at 20 °C as a function of polymer concentration C. Squares corresponds to data from Hammouda and Ho,⁴⁷ and diamonds to data from Cabane and Duplessix.⁴⁸ Full red line: interpolation

formula of Hammouda and Ho.⁴⁷ Full black line are scaling law and dotted lines corresponds locations of the dilute to semidilute crossover according to Cohen et al.¹

At low concentrations (left hand side of the Figure), this length is the radius of gyration of the macromolecules. At high concentrations (right hand side of the figure), it is the mesh size or screening length, which becomes shorter as the concentration is increased and the macromolecules are forced to overlap. The dotted lines correspond to the locations of the dilute to semidilute crossover according to Cohen et al., and it is clearly shown that we have captured this crossover in the used concentration regime i.e. 0-25 mg/ml (to C \approx 5C*).

4.5 Osmotic pressures of silica revisited

In 2011, Jönsson et al.³ presented a comparison between theoretically and experimentally determined osmotic pressures for dispersions consisting of silica particles in various electrolyte solutions. Theoretical equations of state were obtained by performing Monte Carlo simulations in a closed spherical cell on the primitive model level. In the experiments, the osmotic stress technique was used with PEG35 as the stressing polymer. The results are summarized in Figure 10. The mean silica particle radius and salt concentration were 10 nm and 1 mM, respectively. The solid line is the simulated pressure curve. The black squares represent the experimental osmotic pressures for silica dispersions obtained by Persello et al.³⁹ and the black circles represent the osmotic pressures by Chang et al.³⁹ In both of the experiments, the calibration curve by Bouchoux et al.¹⁷ was applied.

As can be seen in Figure 10 there is a systematic discrepancy by roughly a factor of two between the simulated pressures and the experimental data by Persello et al. and Chang et al. As pointed out by Jönsson et al., the deviation between simulations and experiments could be due to an incorrect calibration curve for the stressing polymer, in this case PEG35. Indeed, in this study we have recalculated the experimental data from Persello et al. and Chang et al., applying the EOS,¹ and as clearly shown, an almost perfect match between experimental and theoretical predictions is obtained.



Figure 10. Osmotic pressures in silica dispersions (1mM salt) as a function of the colloidal volume fraction. The solid line corresponds to the simulated osmotic pressures obtained by Monte Carlo simulations in the cell model.³ The black squares and circles correspond to independent experimental osmotic pressures obtained by Persello et al.³⁹ and Chang et al.¹⁵ respectively. They both used the calibration curve for PEG35 established by Bouchoux et al.¹⁷ The red squares and green circles are the corresponding recalculated osmotic pressures using the equation according to Cohen et al.,¹ see equ. 3. Note that in the original paper (Figure 2a),³ the data were plotted on a log-log scale.

5. Conclusions

We have shown by experimental means of several individual groups that the equation of state presented by Cohen et al., based on a scaling relation including one dilute and one semidilute term, gives a good agreement of PEG20 and a fairly good estimation of the osmotic pressures for PEG35 in the range 2-20 wt% (C \approx 35C*) with an discrepancy of maximum \approx 25%, at lower concentration the deviance is \approx 18%. Keeping this in mind, the theoretical one-parameter EOS can be used for osmotic stress measurements, where PEG35 is commonly used as the stressing polymer. On the other hand, the correspondence between experiments and theory for PEG20 is more convincing. Regarding the agreement between theory and experiments, it is all a question about how the data is represented, as shown above. The authors do not state if is the theory or the experiments that is most valid. The results obtained from this study have implications for research groups using this method to probe macromolecular interactions in wide range of systems. Furthermore, we have shown that Monte Carlo simulations and the cell model

can accurately determine the osmotic pressure of silica particles, by validating the results obtained through osmotic stress measurements.

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Highlights (for review)

Equation of state of PEG/PEO in good solvent. Comparison between a one-parameter EOS and experiments.

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- The validity of a single-parameter equation of state has been verified by experiments of several individual groups.
- The theoretical EOS gives a reasonable estimation of the osmotic pressure in the concentration regime 0.1-30 wt% polymer concentration for PEG20 and PEG35.
- Results from this study have implications for correctly probing macromolecular interactions in wide range of systems when applying the osmotic stress method.
- This study shows that the primitive model in combination with the cell mode can be used to accurately determine osmotic pressures of silica dispersions.

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