# Determination of thermodynamic interaction in mixed polymer solutions by a rapid and precise osmotic method. The system dextran/polyvinylpyrrolidone/water

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An accurate and simple osmotic method for determination of thermodynamic interaction in ternary solutions (polymer A/polymer B/solvent) is described. The equipment consists of a membrane osmometer of a special design with small chambers and a pressure-sensing device to monitor the pressure difference across the membrane. A procedure has been developed to measure osmotic pressures up to rather high concentrations in both quasi-binary and quasi-ternary systems. The osmotic method is compared with results from light scattering. Different methods are utilized to extract ordinary virial coefficients as well as mixed virial coefficients from the primary data. The system dextran/polyvinylpyrrolidone/water (two different molecular weights of dextran) was chosen as a model.

**(Keywords: ternary systems; osmotic pressure; light scattering; dextran; polyvinylpyrrolidone)**

## INTRODUCTION

An important class of multicomponent systems is that which contains two or more macromolecular species. Such systems are abundant in nature but do also play an important role in many applications. For instance, when two different macromolecules are mixed in the same solution, the physical properties are often drastically changed. This derives essentially from the usually very marked excluded volume effect found already in a binary macromolecular solution containing only one polymeric species and a solvent. If monomer segment–solvent interactions are favoured, a coiled macromolecule will tend to become partially impenetrable to a neighbouring coil, an effect which increases with increasing molecular weight. Naturally the magnitude of this interaction depends on the chemical properties of the particular solute–solvent pair. If two different types of macromolecules are mixed in the same solution, it is the interplay between the gain in entropy due to mixing and the enthalpy of mixing of the unlike segments that decides the 'mixed' excluded volume. In most cases, especially when the molecular weight is not very low, this 'mixed' excluded volume is larger than the normal binary one. Often the mixed interaction is so strong that it leads to phase separation[1].

One interesting effect deriving from properties of mixtures of two different macromolecular species is the recent discovery of structured, diffusion-controlled, convective flow[2], which gives a considerable enhancement of the mass transport across a diffusion interface.

Although mixed polymer solutions have been investigated for many years, there exist only few studies of their more exact quantitative characteristics[3]. The present study concerns the measurement of virial coefficients for a ternary system containing two macromolecular species in a common solvent by an osmotic technique. These measurements originated because of the need to know more exactly the thermodynamic properties of such systems as an aid to explaining, possibly in a quantitative way, the mechanism of diffusion-controlled, structured, convective flow[2,4–6]. However, the method to be described has proved to be an excellent tool for a precise general characterization of the thermodynamic interaction in macromolecular solutions.

## MEASUREMENTS OF THERMODYNAMIC PROPERTIES OF MULTICOMPONENT POLYMER SOLUTIONS

A variety of methods can be used to determine the thermodynamic properties of polymers in solution. Such methods are measurements of osmotic pressure and light scattering, equilibrium sedimentation and combined velocity sedimentation and diffusion measurements. Each one of those has its advantages or disadvantages, but all are absolute methods. Equilibrium sedimentation is time-consuming but works well at least for higher molecular weights. Velocity sedimentation combined with diffusion measurements is still one of the best methods to determine molecular weights, but it requires fairly complex and heavy equipment. Even here results are obtained only after time-consuming evaluation of extensive primary records. Light scattering is nowadays perhaps the most common direct method in use to determine molecular weights. Its sensitivity increases with molecular weight and usually gives good thermodynamic data if the solutions are treated in such a way that problems with dust and aggregation can be ignored. The most direct thermodynamic method is the measurement of osmotic

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**Table 1** Properties of the fractions

| Sample | Lot no. | $\langle M\rangle_n$ (g mol$^{-1}$) | $\langle M\rangle_w$ (g mol$^{-1}$) | $v_J$ (cm$^3$ g$^{-1}$) | $\nu$ (cm$^3$ g$^{-1}$) |
|---|---|---|---|---|---|
| PVP 360[a] | 125B-1500 | 270000[c] | 1190000[c] | 0.756[d] | 0.1744[e] |
| Dextran T40 | 21945 | 25400[b]<br>30000[c] | 40500[b]<br>38000[c] | 0.604[d] | 0.1417[f] |
| Dextran T70 | 23155 | 39000[b]<br>53000[c] | 75200[b]<br>77000[c] | 0.604[d] | 0.1424[f] |

[a] Molecular weight 360000; no further information given by the manufacturer
[b] Given by the manufacturer
[c] From this study
[d] Ref. 10
[e] 633 nm[14]
[f] 633 nm[15]

pressure. Recent experimental developments[7] have improved its feasibility and made it much more rapid. The span of molecular weights is defined, at the low-molecular-weight end, by the cut-off limit of the membrane used and, at the high end, by the precision of the detection unit. In practice an interval between approximately 10000 up to about 200000 in molecular weight is accessible with reasonable precision.

For the determination of 'mixed' interaction effects a somewhat different reasoning must be used. Here it is the change in measurable properties due to the addition of a third (or fourth, etc.) component that is essential. From this point of view light scattering and osmosis behave quite differently. In light scattering measurements the 'mixed' interaction effect must be calculated as a difference between two large numbers[8] whereas, as will be shown below, the effect comes out directly from adequately designed and very simple osmotic experiments. Furthermore, the problem with dust is avoided. Nor is the cut-off limit of the membrane a limiting factor as long as the low-molecular-weight tail of the sample is not too pronounced. In any case, problems with the low-molecular-weight tail can be avoided by dialysing the samples prior to measurement.

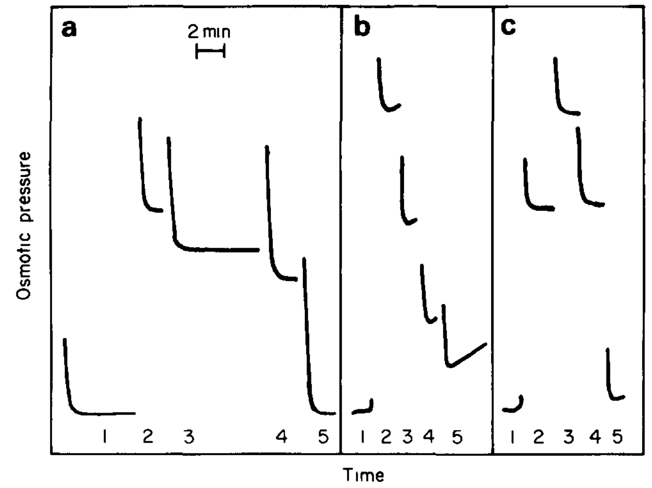

**Figure 1** Recording of osmotic pressure vs. time. The measurements are made from higher to lower concentration. (a) Dextran T70, no leakage; (b) and (c) dextran T40 where some of the polymer leaks through the membrane: 1, 'zero pressure' before samples; 2, 3 and 4, samples of different concentrations; 5, 'zero pressure' after samples

### *Preparation of solutions*

*Materials.* The dextran samples (T40 and T70) were kindly donated by AB Pharmacia, Uppsala, Sweden.

Poly(*N*-vinyl-2-pyrrolidone) (PVP 360) was obtained from Sigma Chemical Co., St. Louis, MO. The polymer fractions were used as received; some data are collected in *Table 1*. Stock solutions were prepared by weight, for PVP taking into account the water content. The water content in PVP was determined by drying to constant weight at 105°C. For dextran the absolute concentration could be determined polarimetrically ($[\alpha]_D^{25} = +200$)[9].

*Dilutions.* These were made by weight, and concentrations (g ml$^{-1}$) were calculated using partial specific volumes, $v_J$ in *Table 1*[10]. Doubly distilled water was used as solvent and sodium azide was added in a concentration of 0.02% to reduce bacterial growth.

### *Osmotic measurements*

Osmotic pressure was measured using a membrane colloid osmometer[7] fitted with Diaflo YM-10 membranes (Amicon Corp., Scientific Systems Division, Danvers, MA). The temperature was 22.0 ± 0.7°C. Equilibrium was reached after 1–2 min. The sample volume was 250 $\mu$l.

The measurements were performed against water (containing 0.02% $NaN_3$) as reference, every third or fourth measurement being a reference measurement with solvent on both sides of the membrane to check 'zero pressure'.

The Diaflo YM-10 membrane was essentially non-permeable for all molecular sizes represented in the PVP and dextran T70 samples. Some leakage of the low-molecular-weight tail of dextran T40 was noticed as a small change in 'zero pressure' (*Figure 1*). When measurements were performed from higher to lower concentrations the change in 'zero pressure' was linear in time and the interpolated 'zero pressure' (baseline pressure) was subtracted. The change in 'zero pressure' was linear in time, when the following procedure was used. The reference compartment was 'saturated' with the low-molecular-weight material by letting the sample with the highest concentration equilibrate with the reference solution until constant pressure was obtained. During the following measurements the low-molecular-weight material leaked out from the reference compartment into the solutions. Since the relative volume sample/reference is large and the equilibration time is short, the changes in concentration and molecular weight distribution were negligible.

### *Light scattering measurements*

The light scattering measurements were performed on a Chromatix KMX-6 low-angle laser light scattering photometer at $\theta = 4.88°$ and $\lambda = 633$ nm and at a temperature of 23 ± 1°C. The refractive index increments at 633 nm are given in *Table 1*. Prior to measurements the samples were filtered through a Millipore filter (pore size 0.22 or 0.45 $\mu$m) directly into the scattering cell. The light scattering data were calculated according to standard practice.

## COMPUTATIONAL ASPECTS

The osmotic pressure of a two-component system can be described by a virial expansion of the form:

$$\pi_J = RT(c_J/\langle M_J\rangle_n + A_{JJ}c_J^2 + A_{JJJ}c_J^3 + \ldots) \qquad (1)$$

where $A_{JJ}$ and $A_{JJJ}$ are the second and third virial coefficients for pure polymer $J$ in a solvent, and $R$ and $T$ have their usual meanings.

For a three-component system (two different polymers in a solvent), the total osmotic pressure can be written as a sum of three parts $\pi_2$, $\pi_4$ and $\pi_{24}$ (using index 2 and 4 for the different polymers):

$$\pi_{\text{tot}} = \pi_2 + \pi_4 + \pi_{24} \qquad (2)$$

where $\pi_{24}$ denotes the contribution due to interaction between species 2 and 4. Part $\pi_{24}$ can be approximated as a polynomial in $c_2$ and $c_4$ according to:

$$\pi_{24} = RT(2A_{24}c_2c_4 + 3A_{224}c_2^2c_4 + 3A_{244}c_2c_4^2 + \ldots) \qquad (3)$$

The numerical factors 2 and 3 derive from a strict definition of interaction parameters[11].

The virial coefficients for the two-component systems were determined from a fit of equation (1) to experimental data. To evaluate the mixed virial coefficients ($A_{24}$, $A_{224}$ and $A_{244}$), the osmotic pressure for dextran and for PVP were calculated from the virial expansion and subtracted from the total osmotic pressure of the three-component solutions. The resulting values of $\pi_{24}$ were fitted to equation (3). All fits were made using the SAS procedure REG[12]. A small error in the virial coefficients of the two-component systems could result in a systematic error in $\pi_{24}$ and hence give large errors in the virial coefficients $A_{24}$, $A_{224}$ and $A_{244}$. To make sure that this was not the case, a fit of the total osmotic pressure $\pi_{\text{tot}}$ was made (using data from both two- and three-component solutions).

The virial coefficients can also be calculated from light scattering experiments; for a two-component system the following relationship holds:

$$Kc_J/\bar{R}_0 = 1/\langle M_J\rangle_w + 2A_{JJ}c_J + 3A_{JJJ}c_J^2 + \ldots \qquad (4)$$

where $K = 2\pi^2n^2v^2/N_A\lambda^4$; here $n$ = refractive index of the solution and $v$ = refractive index increment ($dn/dc$). $\bar{R}_0$ is the Rayleigh ratio of the solution minus that of the solvent in the limit of $\theta \to 0$. The Rayleigh ratio is given by $R_\theta = I_\theta d^2/I_0(1-\cos^2\theta)$ where $I_0$ is the intensity of the incident light and $I_\theta$ is the intensity of the scattered light observed at an angle $\theta$ to the incident light at a distance $d$. Equation (4) is only valid for small particles, or at very low angles ($\theta \to 0$).

For a three-component system the relationship can be written:

$$\begin{aligned}\bar{R}_0/K' = {} & v_2^2M_2c_2 + v_4^2M_4c_4 - 2v_2^2A_{22}M_2^2c_2^2 \\ & - 2v_4^2A_{44}M_4^2c_4^2 - 4v_2v_4A_{24}M_2M_4c_2c_4 \\ & - v_2^2(3A_{222}M_2^2 - 4A_{22}^2M_2^3)c_2^3 \\ & - v_4^2(3A_{444}M_4^2 - 4A_{44}^2M_4^3)c_4^3 \\ & - [v_2^2(3A_{224}M_2^2 - 4A_{24}^2M_2^2M_4) \\ & + 2v_2v_4(3A_{224}M_2M_4 - 4A_{22}A_{24}M_2^2M_4)]c_2^2c_4 \\ & - [v_4^2(3A_{244}M_4^2 - 4A_{24}^2M_2M_4^2) \\ & + 2v_2v_4(3A_{244}M_2M_4 - 4A_{44}A_{24}M_2M_4^2)]c_2c_4^2 \\ & + O(c_2^rc_4^s) \qquad (5)\end{aligned}$$

where $K' = 2\pi^2n^2/N_A\lambda^4$. For the case when $c_2/c_4$ is constant, the second mixed virial coefficient can be calculated from[8]:

$$\frac{K'(c_2+c_4)}{\bar{R}_0} = \frac{1}{v_2^2M_2w_2 + v_4^2M_4w_4} + 2\frac{v_2^2M_2^2w_2^2A_{22} + 2v_2v_4M_2M_4w_2w_4A_{24} + v_4^2M_4^2w_4^2A_{44}}{(v_2^2M_2w_2 + v_4^2M_4w_4)^2}(c_2+c_4) \qquad (6)$$

where $w_J$ is the weight fraction of component $J$ in the polymer mixture. For the two-component systems the experimental data were fitted to equation (4) using the SAS procedure REG[12] and the virial coefficients were calculated. For the three-component systems a curve was fitted to a second-degree polynomial in ($c_2 + c_4$), and then $A_{24}$ was calculated from the initial slope using the virial coefficients from the two-component systems.

## EXPERIMENTAL LAYOUT

Determination of the mixed virial coefficients can be made using either of two methods: (1) keeping the ratio $c_2/c_4$ constant and varying the total concentration $c_2 + c_4$; (2) keeping one concentration constant while varying the other. In method (2) different sets of experiments are performed with different constant concentrations.

For the osmotic measurements the method (2) was chosen since this gives a better visualization of the separate effects from the two different third mixed virial coefficients $A_{224}$ and $A_{244}$ that can be experimentally detected. One sample of polyvinylpyrrolidone and two samples of dextran were used. The properties of the fractions are given in *Table 1*. For each polymer sample the concentration dependence of the osmotic pressure in binary solution was determined, and for each pair of polymers in ternary solution the osmotic pressure was measured for two concentration series. The system dextran T70 (index 2)/PVP 360 (index 4) was investigated in some detail with three series where $c_2$ was kept constant and three series where $c_4$ was kept constant, the

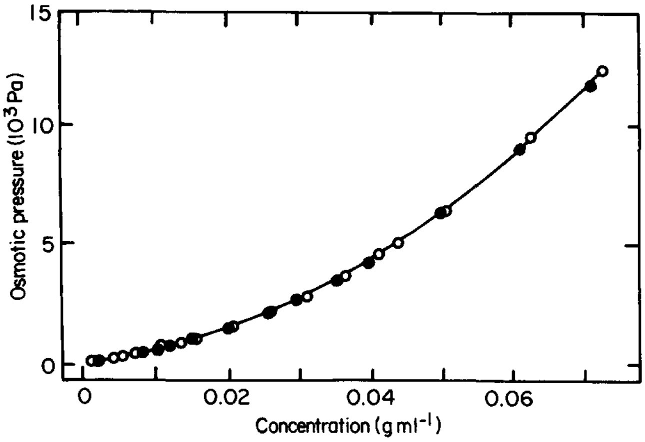

**Figure 2** Osmotic pressure vs. concentration for dextran T70: ○, and ● correspond to two independent series of measurements. Curve fits to experimental data give (see tables for units):

for ○

$$1/\langle M\rangle_n = 1.80(\pm 0.22)\times 10^{-5}$$
$$A_{22} = 6.17(\pm 0.97)\times 10^{-4}$$
$$A_{222} = 1.38(\pm 0.99)\times 10^{-3}$$

for ●

$$1/\langle M\rangle_n = 1.87(\pm 0.19)\times 10^{-5}$$
$$A_{22} = 5.70(\pm 0.85)\times 10^{-4}$$
$$A_{222} = 1.71(\pm 0.89)\times 10^{-3}$$

for both series

$$1/\langle M\rangle_n = 1.83(\pm 0.17)\times 10^{-5}$$
$$A_{22} = 5.94(\pm 0.75)\times 10^{-4}$$
$$A_{222} = 1.55(\pm 0.77)\times 10^{-3}$$

aim being to examine whether the concentration of the constant component affected the results.

In the light scattering measurements method (1) was chosen (for a discussion of different methods see ref. 8) and the two systems dextran T70/PVP 360/water and dextran T40/PVP 360/water were investigated. For each system two concentration series were followed with $c_2/c_4 = 10$ and $c_2/c_4 = 2.5$. The total concentration $c_2 + c_4$ was varied from 0 up to 0.025 g ml$^{-1}$. Both series were chosen to have $c_2/c_4 > 1$, since PVP gives a higher scattering intensity than dextran and it is important to have at least approximately similar scattering contributions from the two components to lower the error in the determination of $A_{24}$ (ref. 13).

## RESULTS AND DISCUSSION

### *Two-component systems*

The aim of this investigation was to obtain accurate thermodynamic information over an extended concentration interval. Hence osmotic measurements have been made up to relatively high concentrations. This poses certain experimental problems since the measurement accuracy depends on the absolute pressure and since different ranges must be set on the pressure recorder. The accuracy was tested in various ways. For one thing the continuity observed in the recorded osmotic pressure as a function of concentration gives some indication, as does also the general scattering of points. The most crucial test performed was the two completely independent measurement series presented in *Figure 2*. This figure indicates a very high reproducibility, a very good continuity over the detector ranges and a very good definition of the origin, i.e. zero pressure for zero concentration.

The numerical analysis of experimental data can normally be done in the form of a polynomial approximation defining the virial expansion coefficients. An essential question is then how many of these coefficients can reliably be numerically extracted. Various procedures for fitting the data were applied and they all showed that the virial coefficients of fourth and higher degrees were equal to zero within experimental error, for the systems studied.

Low-molecular-weight material in the sample could result in leakage of the smaller molecules through the membrane. The extent of such leakage is determined by the low-molecular-weight tail of the molecular-weight distribution of the sample together with the cut-off limit of the membrane. Leakage of this type could affect the measurements in different ways. For example, the leakage of low-molecular-weight molecules will change the molecular-weight distribution of the sample and the proportion of molecules leaking through the membrane need not be constant over the concentration interval. Systematic errors in the measurements would then arise and hence affect the coefficients determined. A leakage of low-molecular-weight material is easily discovered since no constant pressure can be determined and the 'zero pressure' changes during the measurements. For all systems studied[10] only one sample, namely dextran T40, gave this problem. A small leakage of this kind can be corrected for using the method described in the 'Experimental' part.

In order to get an idea of the errors connected with such leakage, a number of experiments were performed for the dextran T40 system. These experiments give a general procedure for how to handle moderate leakage and still get acceptable results. Using the method where the solutions were measured from high to low concentration, after equilibration of the reference solution with the solution of highest concentration, the low-molecular-weight material in the reference solution would leak into the sample solution. If on the other hand the measurements were performed from low to higher concentration, the low-molecular-weight material would leak out of the sample solution, giving a systematic change in the opposite direction. Solutions of five different concentrations were taken (each solution was measured nine times) from low to high concentration, from high to low concentration and in a random order. Independent of the measuring method the maximum difference between two individual measurements on the same solution was <3%.

To illustrate the change in 'zero pressure' and the corrections connected with the interpolation method, the 'zero pressure' was recorded for different pressure ranges during the leakage of low-molecular-weight material out of the reference compartment (*Figure 3*). The solvent in the measuring compartment was changed approximately at the same intervals as the samples during the measurements. The change in 'zero pressure' is only noticeable for the lowest pressure ranges, which corresponds to measurements made on solutions with concentrations lower than 0.008 g ml$^{-1}$ (compare with

*Table 2*). Hence, the corrections are made only for the four most dilute solutions and therefore will not affect the virial coefficients to any large extent.

For the three-component systems the leakage effect would be observed only on still more dilute solutions since the pressure measured is higher than for the two-component solutions.

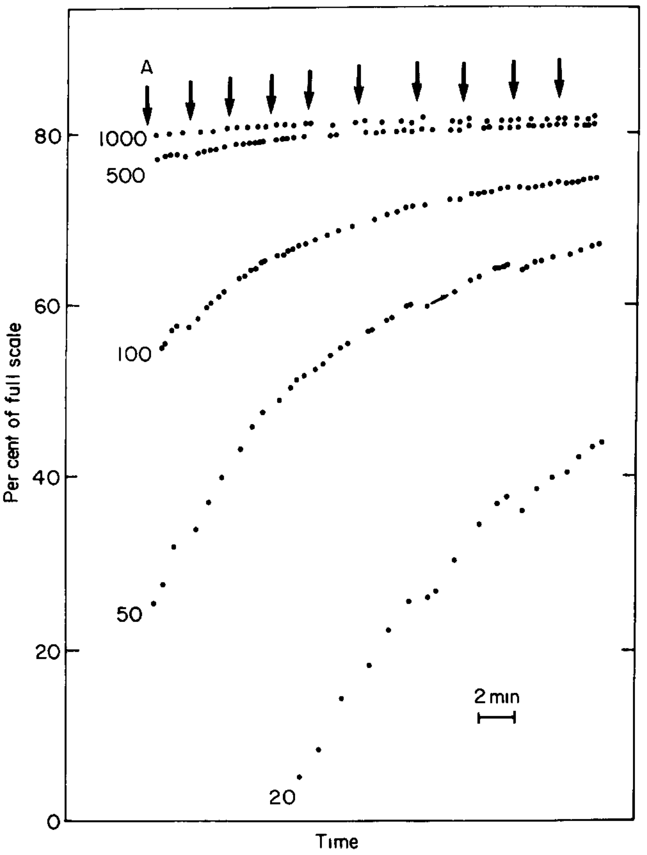

**Figure 3** Recording of 'zero pressure' for different sensitivity ranges (20–1000) after equilibration of the solvent in the reference compartment with 5% dextran T40 solution in the measuring compartment. Arrows indicate the change to fresh solvent in the measuring compartment. Arrow at A indicates start of experiment

**Table 2** Pressure ranges on the pressure recorder

| Range | Corresponds to pressure interval (Pa) | Corresponds to conc. T40 (g ml$^{-1}$) | Maximum reading accuracy (Pa) |
|---|---|---|---|
| 2500 | 6500–16000[a] | 0.045– | 36–55 |
| 1000 | 3300–6500[a] | 0.03–0.045 | 15–22 |
| 500 | 650–3300[a] | 0.008–0.03 | 7–11 |
| 100 | 330–650[a] | 0.004–0.008 | 1.5–2.2 |
| 50 | 0–330[a] | 0–0.004 | 0.7–1.1 |

[a] Full scale on pressure recorder at given range

In the light scattering measurements each solution was measured three times to reduce the errors. *Figure 4* shows the results for dextran T40. The virial coefficients resulting from the curve fit are given in *Table 3*. The results are in good agreement with the results from the osmotic measurements and the 95% confidence intervals are somewhat smaller than for osmotic measurements. This could be partly due to the greater number of experiments in the light scattering than in the osmotic measurements. From a practical point of view the two methods are equally good for determining virial coefficients of two-component systems. However, for three-component systems the osmotic method is to be preferred as will be shown below.

### *Three-component systems*

For osmotic measurements on three-component systems, it is convenient to have two concentrations series to be able to determine both the second and the third mixed virial coefficients. The calculation of the higher-order coefficients is simplified if the osmotic pressure is known as a function of the concentration of each of the solutes, in the presence of a constant amount of the other. In order to determine the most favourable combination of concentrations, the system dextran T70/PVP 360 was investigated in detail. It is apparent from the results (*Table 4*) that, while it is difficult to get an estimate of both of the third mixed virial coefficients when the concentration dependence of the osmotic pressure is known for only one solute, all three mixed virial coefficients can be obtained readily if it is known for both. A high concentration of the constant component could give rather large errors if the

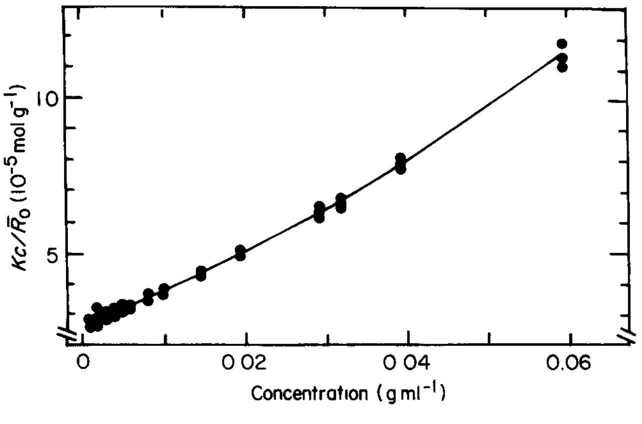

**Figure 4** The concentration dependence of $Kc/\bar{R}_0$ for dextran T40 with line of best fit

**Table 3** Virial coefficients of two-component systems determined by osmometry and light scattering

| | $1/M$ ($10^{-5}$ mol g$^{-1}$) | | $A_{JJ}$ ($10^{-4}$ mol cm$^3$ g$^{-2}$) | | $A_{JJJ}$ ($10^{-3}$ mol cm$^6$ g$^{-3}$) | |
|---|---|---|---|---|---|---|
| Sample | Osmom. | LS | Osmom. | LS | Osmom. | LS |
| PVP 360[a] | 0.373 (±0.068)[c] | 0.084 (±0.008) | 6.18 (±0.90) | 2.74 (±0.18) | 14.0 (±2.7) | 15.7 (±0.9) |
| Dextran T40[b] | 3.37 (±0.16) | 2.61 (±0.07) | 5.97 (±0.70) | 5.04 (±0.39) | 1.32 (±0.71) | 2.77 (±0.46) |
| Dextran T70[b] | 1.87 (±0.19) | 1.30 (±0.04) | 5.71 (±0.85) | 4.01 (±0.25) | 1.71 (±0.89) | 3.31 (±0.29) |

[a] $0 < c < 0.03$ g cm$^{-3}$
[b] $0 < c < 0.07$ g cm$^{-3}$
[c] Values in parentheses are ($t_{0.975} \times$ std. error) to give 95% confidence interval

**Table 4** Virial coefficients of the system dextran T70 (component 2)/PVP 360 (component 4) determined by osmometry for different concentration series

| Series | $c_2$ (g cm$^{-3}$) | $c_4$ (g cm$^{-3}$) | $A_{24}$ ($10^{-4}$ mol cm$^3$ g$^{-2}$) | $A_{224}$ ($10^{-3}$ mol cm$^6$ g$^{-3}$) | $A_{244}$ ($10^{-3}$ mol cm$^6$ g$^{-3}$) |
|---|---|---|---|---|---|
| Low | 0–0.06 | 0.005 | 8.22 (±0.92)[a] | 2.6 (±1.1) | 5.1 (±3.6) |
| | 0.01 | 0–0.02 | | | |
| Medium | 0–0.04 | 0.01 | 7.67 (±0.82) | 0.7 (±1.4) | 9.5 (±2.3) |
| | 0.02 | 0–0.02 | | | |
| High | 0–0.03 | 0.02 | 5.84 (±1.28) | 5.4 (±2.1) | 7.6 (±3.3) |
| | 0.03 | 0–0.02 | | | |
| All | All series above | | 7.32 (±0.70) | 2.9 (±1.0) | 6.3 (±1.9) |
| $c_2$ const. | 0.01 | 0–0.02 | 7.82 (±0.67) | 2.7 (±2.2) | 6.9 (±1.6) |
| | 0.02 | 0–0.02 | | | |
| $c_2$ const. | 0.01 | 0–0.02 | 8.34 (±0.74) | 0.2 (±1.3) | 6.3 (±2.0) |
| | 0.03 | 0–0.02 | | | |
| $c_4$ const. | 0–0.06 | 0.005 | 9.26 (±1.26) | 2.4 (±1.2) | 9.7 (±7.4) |
| | 0–0.04 | 0.01 | | | |
| $c_4$ const. | 0–0.06 | 0.005 | 6.96 (±1.48) | 4.5 (±1.7) | 4.8 (±4.1) |
| | 0–0.03 | 0.02 | | | |

[a] Values in parentheses are ($t_{0.975}$ × std. error) to give 95% confidence interval

**Table 5** Comparison of data[a] for the three-component systems as determined by osmometry according to methods (a) and (b)[b]

| | Dextran T40/PVP 360[c] | | Dextran T70/PVP 360[d] | |
|---|---|---|---|---|
| | (a) | (b) | (a) | (b) |
| $1/M_2$ (mol g$^{-1}$) | 0.337 (±0.016)[e] | 0.339 (±0.009) | 0.187 (±0.019) | 0.190 (±0.016) |
| $A_{22}$ (mol cm$^3$ g$^{-2}$) | 5.97 (±0.70) | 5.91 (±0.41) | 5.71 (±0.85) | 5.59 (±0.73) |
| $A_{222}$ (mol cm$^6$ g$^{-3}$) | 13.2 (±7.1) | 14.0 (±4.2) | 17.1 (±8.9) | 18.5 (±7.7) |
| $1/M_4$ (mol g$^{-1}$) | 0.037 (±0.007) | 0.030 (±0.022) | 0.037 (±0.007) | 0.088 (±0.040) |
| $A_{44}$ (mol cm$^3$ g$^{-2}$) | 6.18 (±0.90) | 7.7 (±2.6) | 6.18 (±0.90) | 1.08 (±5.05) |
| $A_{444}$ (mol cm$^6$ g$^{-3}$) | 140 (±27) | 85 (±79) | 140 (±27) | 370 (±150) |
| $A_{24}$ (mol cm$^3$ g$^{-2}$) | 8.65 (±0.60) | 8.4 (±1.0) | 7.32 (±0.70) | 6.7 (±1.2) |
| $A_{224}$ (mol cm$^6$ g$^{-3}$) | 23.8 (±8.4) | 27 (±14) | 29 (±10) | 30 (±15) |
| $A_{244}$ (mol cm$^6$ g$^{-3}$) | 39 (±18) | 44 (±28) | 63 (±19) | 88 (±29) |

[a] Values in the table should be multiplied by $10^{-4}$

[b] Method (a): $\pi_2$ and $\pi_4$ fitted to equation (1), $\pi_{24}$ calculated and fitted to equation (3). Method (b): $\pi_{tot}$ fitted to equation 2 (equation (1) and equation (3))

[c] Concentration series: $0<c_2<0.05$ g cm$^{-3}$, $c_4=0.005$ g cm$^{-3}$; $c_2=0.001$ g cm$^{-3}$, $0<c_4<00025$ g cm$^{-3}$

[d] Concentration series: 'All', see *Table 3*

[e] Values in parentheses are ($t_{0.975}$ × std. error) to give 95% confidence interval

change in pressure due to the variation of the other component is small compared with the total pressure, i.e. a small variation in the pressure is measured on a scale of low sensitivity. For the different combinations of measurement series in this study, there are no systematic differences between high and low concentrations of the constant component.

Two methods of computation were used as a test for systematic errors in the method of evaluation. Method (a) is a fit of the mixed osmotic pressure after subtracting the calculated osmotic pressure from dextran and PVP. Method (b) is a fit of the total osmotic pressure for both the two-component and the three-component systems at the same time. The two methods give the same results within experimental error. The results are given in *Table 5*. Method (b) sometimes gives very large errors in the virial coefficients for the high-molecular-weight component. This is probably due to the fact that the data analysis is made without using weighted data and the high pressure of the low-molecular-weight component is measured with much lower sensitivity than the relatively low pressure of the high-molecular-weight component. *Figure 5* shows the different contributions to the total osmotic pressure for the system dextran T40/PVP 360.

In the light scattering experiments the second mixed virial coefficient $A_{24}$ is calculated from the initial slope of $K'(c_2+c_4)/\bar{R}_0$ vs. $(c_2+c_4)$; see *Figure 6*. Since the third mixed virial coefficients are of importance even at very low concentrations (*Figure 7*), the initial slope was determined using a polynomial in $(c_2+c_4)$. The values of the second mixed virial coefficients (*Table 6*) are in good agreement with those determined by osmometry. Higher mixed virial coefficients than the second cannot reliably be extracted from the light scattering data.

## CONCLUSIONS

Osmotic measurements constitute a convenient and accurate method for determination of thermodynamic properties in polymeric systems, basically since it is a direct measurement of the water activity in the solution. It is definitely to be preferred to light scattering in the case of multicomponent systems. Osmometry has its highest sensitivity at low molecular weights; light scattering, on the other hand, has its highest sensitivity at high molecular weights. Osmotic measurements give good accuracy in determining the virial coefficients also for relatively high-molecular-weight samples, since the pressure measured is relatively high for high concentrations, especially because of the non-ideality

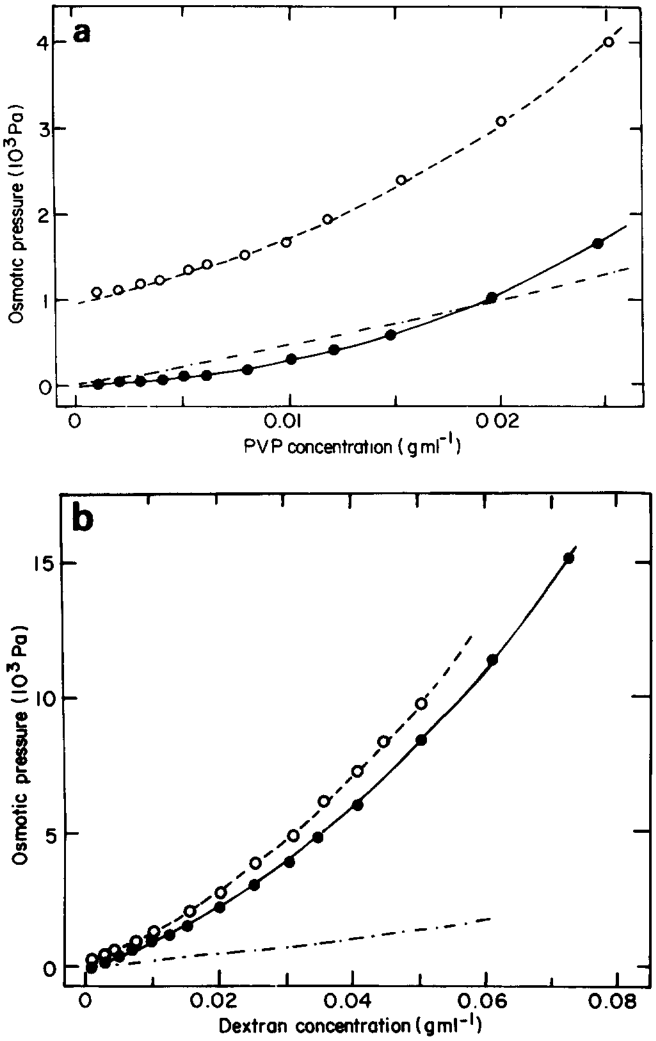

**Figure 5** (a) Osmotic pressure of the system dextran T40/PVP 360 vs. concentration of PVP with line of best fit. Curves give the pressure calculated from the virial coefficients: —●—, $\pi_4$ vs. $c_4$; — ○ —, $\pi_{tot}$ vs. $c_4$; —·—·—, $\pi_{24}$ vs. $c_4$. (b) Osmotic pressure of the system dextran T40/PVP 360 vs. concentration of dextran. Curves give the pressure calculated from the virial coefficients: —●—, $\pi_2$ vs. $c_2$; — ○ —, $\pi_{tot}$ vs. $c_2$; —·—·—, $\pi_{24}$ vs. $c_2$

terms. Light scattering measurements at high concentrations or for very large molecules could be difficult to interpret due to multiple scattering effects and difficulties with dust, even after extensive filtration. Problems could also arise in light scattering measurements on three-component systems for solutions close to phase separation. The results presented here show that accurate thermodynamic data can be obtained over extended concentration intervals for both binary and ternary macromolecular systems.

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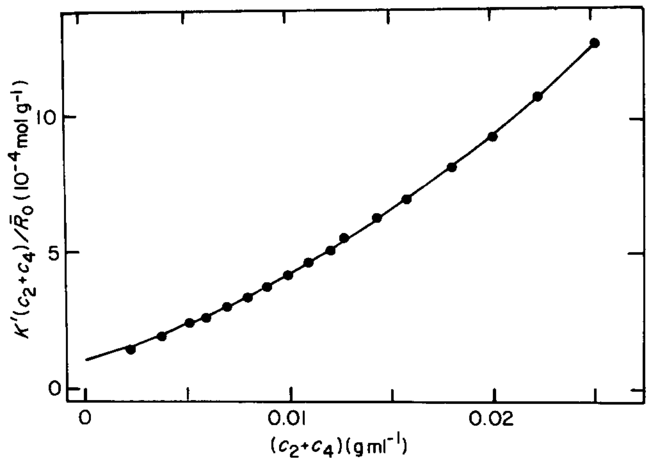

**Figure 6** Light scattering measurements of the three-component system dextran T40/PVP 360/water, concentration series 2/5, with line of best fit

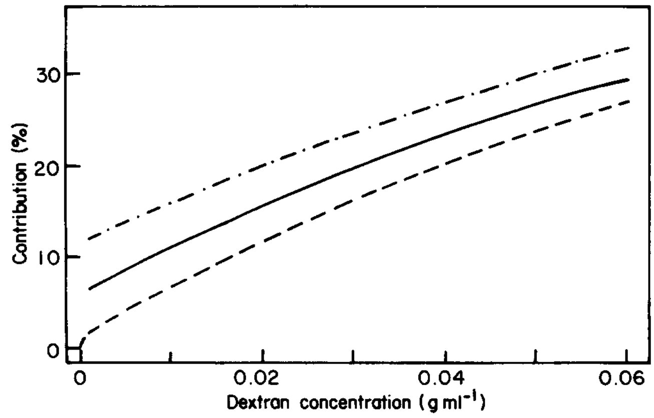

**Figure 7** Relative contribution of the third mixed virial coefficients to the total mixed osmotic pressure ($\pi_{24}$) vs. dextran concentration for the system dextran T70/PVP 360: -----, $c_4$=0.001 g ml$^{-1}$; ———, $c_4$=0.005 g ml$^{-1}$; —·—·—, $c_4$=0.010 g ml$^{-1}$

**Table 6** Virial coefficients of the three-component systems determined by light scattering

| System (component 2/component 4) | Series | $A_{24}$ ($10^{-4}$ mol cm$^3$ g$^{-2}$) |
|---|---|---|
| Dextran T40/PVP 360 | 1/10 | 7.7 |
| | 2/5 | 8.1 |
| Dextran T70/PVP 360 | 1/10 | 5.0 |
| | 2/5 | 6.4 |

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