Biophysical Chemistry, 33 (1989) 115–125
Elsevier

BPC 01341

Evaluation of nonideality from gel chromatographic partition coefficients

A technique with greater versatility than equilibrium dialysis

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Received 26 April 1988
Revised manuscript received 31 October 1988
Accepted 14 November 1988

Thermodynamic nonideality; Interaction coefficient; Gel chromatography; Partition coefficient; Excluded volume

Frontal gel chromatography has been used to measure partition coefficients which enable a quantitative evaluation of the thermodynamic nonideality of small solutes generated by the presence of high concentrations of macromolecular solutes. Equivalence of results obtained by the present method and by equilibrium dialysis is demonstrated in a comparison of results for dextran sulfate-NaCl and dextran-sorbitol systems. Interaction coefficients obtained for dextran-sorbitol and protein-polyethylene glycol 4000 systems yield results which are in reasonable agreement with those predicted on the statistical-mechanical basis of excluded volume. Because of its greater versatility in regard to the range of systems that may be studied, the frontal gel chromatographic procedure is likely to be of particular value for the quantitative characterization of thermodynamic nonideality arising from excluded volume effects in concentrated mixtures of macromolecular solutes.

1. Introduction

A major requirement for the understanding of physiological function is the quantitative assessment of macromolecular interactions in concentrated polymer solutions in which most biological phenomena occur. These interactions arise either from thermodynamic interactions (e.g., steric exclusion effects due to the expanded molecular domains of the macromolecules) or from electrostatic interactions. Thus, the composition in the local microenvironment is influenced by the nature and concentration of the polymer species. Evaluation of interaction coefficients is therefore essential to the understanding of physiological transport processes [1–6] and to assessment of the likely extent and rates of interactions under physiological conditions [7–14].

Until recently, the effects of high concentrations of polymer on the partitioning of a small solute between a polymer solution and a polymer-free solution have been measured by equilibrium dialysis in systems such as dextran-sorbitol and dextran sulfate-NaCl [5,15]. However, this technique has limitations in regard to the availability of membranes impermeable to low molecular weight polymers (M_r 10000 or less), especially when studied at high concentrations (> 10%, w/w), and also with respect to the long times (10–20 days) apparently required for the attainment of equilibrium [15].

In the present investigation, a frontal gel chromatographic technique, where the gel (Fractogel TSK HW 40) replaces the membrane, is described

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which provides a faster and more convenient means of obtaining the required interaction coefficients. Furthermore, greater versatility accrues from the fact that the method may also be applied to systems in which the larger solute is not completely excluded from the gel phase. These aspects of the gel chromatographic procedure are illustrated with studies of the dextran-sorbitol and dextran sulfate-NaCl systems previously studied by equilibrium dialysis [5,15]. Interaction coefficients obtained by the gel chromatographic technique for globular protein-polyethylene glycol systems are also compared with those deduced from phase separation studies [16].

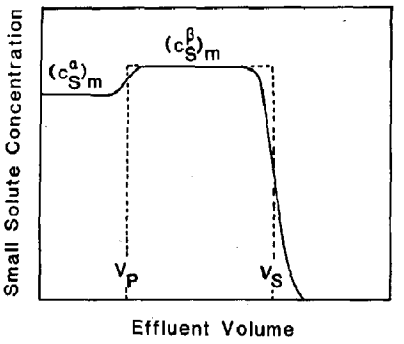

Fig. 1. Schematic representation of the trailing elution profile for small solute in frontal gel chromatography of a mixture of macromolecule (P) and small solute (S). V_P and V_S refer to the elution volumes of polymer and small solute, respectively; $(c_S^\alpha)_m$ and $(c_S^\beta)_m$ denote the respective concentrations of small solute in the α and β regions of the mobile phase.

2. Theory

2.1. Analogy between gel chromatography and equilibrium dialysis

In equilibrium dialysis of a mixture of uncharged macromolecular solute, P, and small solute, S, the final state may be described in terms of one phase with solute concentrations c_P^a and c_S^a, and a second phase with concentration c_S^b of small solute. A similar situation is observed in the trailing elution profile generated by frontal gel chromatography of such a mixture [17]. After addition of sufficient mixture to the column to ensure identity of the applied and effluent compositions (c_P^α, c_S^α), elution of the column with solvent yields a trailing elution profile of the form shown schematically in fig. 1. The concentrations of P and S remain at their initial values (c_P^α, c_S^α) until V_P, the elution volume of P, at which stage the concentration of small solute attains a new value, c_S^β. This plateau is maintained until V_S, the elution volume of small solute, at which stage the concentration decreases to zero.

Since the area under the elution profile for S corresponds to w_S, the amount of small solute on the mixture-saturated column, it follows that

$$w_S = V_P(c_S^\alpha)_m + (V_S - V_P)(c_S^\beta)_m \quad (1)$$

where the subscript m is used to signify that the elution profile reflects solute concentration in the mobile phase at the exit plane of the column. An alternative expression for w_S is obtained by considering the mixture-saturated column in terms of a void (mobile-phase) volume with solute concentration $(c_S^\alpha)_m$ and a gel-phase volume with solute concentration $(c_S^\alpha)_g$. In these terms conservation of solute requires that

$$w_S = V_0(c_S^\alpha)_m + (V_t - V_0)(c_S^\alpha)_g \quad (2)$$

where V_0 refers to the void volume of the column, and the gel-phase volume is expressed as the difference between V_0 and the total accessible volume, V_t. Combination of eq. 2 with eq. 1 and the expressions for elution volumes in terms of Gelotte [18] distribution coefficients σ_i, namely, $V_i = V_0 + \sigma_i(V_t - V_0)$, yields the relationship

$$(c_S^\alpha)_g = \sigma_S(c_S^\beta)_m + \sigma_P[(c_S^\alpha)_m - (c_S^\beta)_m] \quad (3)$$

The situation equivalent to equilibrium dialysis is achieved by setting $\sigma_P = 0$ to conform with the confinement of macromolecular component to the one phase (mobile phase in the chromatographic context), and setting $\sigma_S = 1$ to allow small solute complete access to the second (gel) phase. With these substitutions eq. 3 simplifies to

$$(c_S^\alpha)_g = (c_S^\beta)_m \quad (4)$$

which signifies that under those conditions the concentration of S in the trailing (β) plateau of

the elution profile is a direct measure of its concentration in the gel phase of the α-plateau. This conclusion was reached previously [19] in relation to the operation of a Donnan effect in gel chromatography, the condition $\sigma_S = 1$ being implicit in the earlier presumption that $(c_S^{\beta})_m = (c_S^{\beta})_g$. The analogy with equilibrium dialysis is hence that concentrations in the α-plateau region of the elution profile, $(c_P^{\alpha})_m$ and $(c_S^{\alpha})_m$, correspond to c_P^a and c_S^a, while the concentration of S in the β-plateau, $(c_S^{\beta})_m$, is equivalent to c_S^b, its concentration on the diffusate side of a semipermeable membrane.

In addition to providing a means of determining $(c_S^{\alpha})_g$ under those relatively restrictive conditions, eq. 3 also allows the estimation of this quantity under conditions where macromolecular solute is incompletely excluded ($\sigma_P \neq 0$), and/or where accessibility of the gel phase to S is incomplete ($\sigma_S < 1$). Clearly, the only experimental restriction to the determination of $(c_S^{\alpha})_g$ by the application of eq. 3 is the requirement that σ_P and σ_S differ sufficiently for the trailing elution profile to contain a readily identifiable and hence quantifiable β-plateau region.

2.2. *Thermodynamic activity of small solute in the gel phase*

Since interaction coefficients are to be obtained by equating the chemical potentials of small solute in the mobile and gel phases of the α-plateau region, it is first desirable to establish the most convenient way to account for the effect of the gel matrix on the thermodynamic activity of S in the gel phase. For this purpose, it is simplest to consider the α-plateau region in a gel chromatographic experiment performed on S alone. On the basis of composition-dependent activity coefficients assessed by the statistical-mechanical application of the excluded volume concept [20,21], the respective thermodynamic activities, a_S, of small solute in the mobile and gel phases are

$$(a_S^{\alpha})_m = (c_S^{\alpha})_m \exp\left[\alpha_{SS}(c_S^{\alpha})_m\right] \quad (5a)$$

$$(a_S^{\alpha})_g = (c_S^{\alpha})_g \exp\left[\alpha_{SS}(c_S^{\alpha})_g + \alpha_{SG}(c_G^{\alpha})_g\right] \quad (5b)$$

where α_{SS} is the second virial coefficient describing nonideality due to self-self interaction of S, and α_{SG} is the corresponding virial coefficient for nonideality of S imposed by the presence of gel matrix at concentration c_G: third and higher virial terms are thus neglected. The fact that the two phases are in partition equilibrium allows these two activities to be equated, whereupon it follows that

$$(c_S^{\alpha})_g/(c_S^{\alpha})_m = \exp\left\{\alpha_{SS}\left[(c_S^{\alpha})_m - (c_S^{\alpha})_g\right] - \alpha_{SG}(c_S^{\alpha})_g\right\} \quad (6)$$

However, the left-hand side of eq. 6 is an alternative definition of the solute distribution coefficient, σ_S [22,23], which thus takes into account all thermodynamic nonideality due to the presence of small solute – not only the nonideality due to self-self interaction but also that arising from interaction of S with gel matrix.

2.3. *Evaluation of the thermodynamic interaction coefficient for an inert macromolecule and a small solute*

For an experiment in which the α-plateau of the elution profile contains both S and a macromolecular solute, P, the thermodynamic activities of S in the two phases may be written as

$$(a_S^{\alpha})_m = (c_S^{\alpha})_m \exp\left[\alpha_{SS}(c_S^{\alpha})_m + \alpha_{SP}(c_P^{\alpha})_m\right] \quad (7a)$$

$$(a_S^{\alpha})g = (c_S^{\alpha})_g \exp\left[\alpha_{SS}(c_S^{\alpha})g + \alpha_{SG}(c_G^{\alpha})_g + \alpha_{SP}(c_P^{\alpha})_g\right] \quad (7b)$$

By including a virial coefficient term for the S-P interaction in both phases, we cover the possibility that the macromolecular solute may also partition into the gel phase, where its concentration is related to its mobile-phase counterpart by the relationship $(c_P^{\alpha})_g = \sigma_P(c_P^{\alpha})_m$. When combining these two activities on the ground of equal chemical potentials in the two phases, advantage may be taken of eq. 6 to account for thermodynamic effects due to small solute. The resulting expression, namely,

$$(c_S^{\alpha})_g/(c_S^{\alpha})_m = \sigma_S \exp\left\{\alpha_{SP}/\left[(1-\sigma_P)(c_P^{\alpha})_m\right]\right\} \quad (8)$$

is then converted to a more convenient form for analysis of frontal gel chromatographic data by replacing $(c_S^{\alpha})_g$ by eq. 3 to obtain

$$\alpha_{SP} = \frac{\ln[R - (\sigma_P/\sigma_S)(R-1)]}{(c_P^{\alpha})_m(1-\sigma_P)} \quad (9)$$

where $R = (c_S^{\beta})_m/(c_S^{\alpha})_m$ is the reciprocal of the corresponding equilibrium dialysis parameter, c_S^a/c_S^b, termed the partition coefficient. In the event that the macromolecular solute is excluded from the gel phase ($\sigma_P = 0$), this relationship for evaluating the second virial coefficient describing the mutual effects of polymer and small solute on each other's thermodynamic nonideality simplifies to

$$\alpha_{SP} = [\ln R]/(c_P^{\alpha})_m \quad (10)$$

The finding that α_{SP} follows directly from the ratio of small solute concentrations in the two phases is entirely in accord with the situation for equilibrium dialysis, and is thus the conclusion that would have been reached by simply regarding the gel as a second liquid phase with which to achieve partition equilibrium. This demonstration that eq. 10 is also obtained when allowance is made for the thermodynamic effects arising from the presence of gel matrix eliminates any cause for concern that the analogy between equilibrium dialysis and gel chromatography may rely upon total disregard of the matrix as a component of the gel phase.

3. Experimental

3.1. Materials

Dextran polymers (T10, T20, T40, T70 and T200) and dextran sulfate ($M_n = 45\,600$ and $M_w = 74\,800$, estimated from parental dextran molecular weight) were supplied by Pharmacia (Sweden). Bovine serum albumin (fraction V), bovine thyroglobulin and polyethylene glycol 4000 (PEG-4000) were obtained from Sigma (St. Louis, MO). Sorbitol was a product of BDH (Poole, U.K.); and other chemicals were of reagent grade.

Radioactively labelled materials (^3H_2O, [^{14}C]-PEG-4000, ^{22}NaCl and Na^{36}Cl) were purchased from New England Nuclear (Boston, MA).

Fractogels TSK HW 40 and HW 55 fine (Merck, Darmstadt, FRG) were packed in an Omnifit column (Amersham, U.K.).

3.2. Preparation of solutions

3.2.1. Macroion and uniunivalent electrolyte

Dextran sulfate (10–50 mg/ml) was dissolved in NaCl (0.02–0.10 M) supplemented with trace quantities of either ^{22}Na-labelled NaCl (10 000 dpm/ml) or ^{36}Cl-labelled NaCl (30 000 dpm/ml). The concentration of the macroion, c_e (equiv./l.), was calculated from the electroneutrality requirement that $c_{Na^+} = c_{Cl^-} + c_e$, where c_{Na^+} and c_{Cl^-} represent the concentrations of the respective electrolyte ions in the presence of dextran sulfate: these values were obtained from the frontal gel chromatography experiment performed on solutions of identical concentration containing either ^{22}Na or ^{36}Cl (see section 4.1).

3.2.2. Dextran-small neutral solute

Solutions of dextran polymers (T10, T20, T70 and T200) were prepared by weight in 0.1 M NaCl containing sorbitol (5–50 mg/ml) and [^{14}C]sorbitol 20 000 dpm/ml); tritiated water (20 000 dpm/ml) was also added in order to determine the total accessible volume of the column, V_t.

The interaction coefficients for dextran and NaCl were evaluated using dextran T40 (38.5 mg/ml) dissolved in 0.1 M NaCl supplemented with trace amounts of either ^{22}Na-labelled or ^{36}Cl-labelled NaCl (20 000 dpm/ml) in order to assess the contribution of the polymer to the entropic interaction in the dextran sulfate-NaCl system previously studied.

3.2.3. Protein-polyethylene glycol

Bovine serum albumin or thyroglobulin (10–30 mg/ml) and PEG-4000 (1 mg/ml) were dissolved in 0.1 M NaCl buffered with 0.03 M Tris-HCl buffer (pH 7.4), the mixture being supplemented with trace quantities (20 000 dpm/ml) of [^{14}C]PEG-4000 and tritiated water.

3.3. Analytical methods

Concentrations of the dextrans and dextran sulfate were measured by optical rotation at 589 nm in a Perkin-Elmer model 141 polarimeter: $[\alpha] = 0.914$ for a 4.3% (w/w) solution of dextran in a 1 cm cell, and $[\alpha] = 0.53$ for a 5% (w/w) solution of dextran sulfate. Concentrations were then converted to a molar scale on the basis of a partial specific volume of 0.60 ml/g for dextran [20] and the manufacturer's value of the number-average molecular weight (M_n). Concentrations of the proteins were estimated spectrophotometrically (Varian Techtron model 634) at 280 nm on the basis of absorption coefficients ($A^{1\%}_{1cm}$) of 6.6 [24] and 10.5 [25] for albumin and thyroglobulin, respectively. Radioactively labelled small solutes were measured either in a liquid scintillation counter (LKB 1215 Rack Beta) using a Triton-based scintillation cocktail [26], or in a gamma counter (LKB Multigamma). In some experiments the elution profile for Na^+ was monitored by atomic absorption spectroscopy (Varian Techtron model 1000).

3.4. Measurement of partition coefficients by frontal gel chromatography

Samples to be analyzed were filtered through a 0.45 μm Millipore filter prior to gel chromatography. In the initial studies of polymer-small solute systems, mixtures prepared in 0.1 M NaCl were applied to a column (1.2 × 48 cm) of Fractogel TSK HW 40 at 18 ml/h: 0.35-ml fractions were collected. After application of a sufficient volume (approx. 40 ml) of sample to ensure that the elution profile contained a plateau region in which the composition matched that of the applied mixture [17], elution with 0.1 M NaCl was continued to generate the trailing elution profile.

In subsequent studies a smaller column (0.66 × 40 cm) of Fractogel TSK HW 40 was employed at a flow rate of 3.6 ml/h, the applied sample volume being decreased accordingly (8–9 ml). Fractions (0.08 ml) were collected in preweighed disposable vials, the pre- and post-collection weight data being stored in an IBM personal computer (PC-XT) linked to a Mettler AE 140 balance. All calculations, data tabulation and plotting of elution profiles were performed using Lotus 123.

Partition coefficients, $(c^{\alpha}_S)_m/(c^{\beta}_S)_m$, were calculated from the ratios of the two plateaux in the radioactivity profiles of the small solutes.

3.5. Evaluation of thermodynamic interaction coefficients

Interaction coefficients were calculated from the partition coefficients ($1/R$) of the small solutes by means of eq. 9, the distribution coefficients of polymer (σ_P) and small solute (σ_S) being based on void (V_0) and total (V_t) volumes obtained as elution volumes of dextran T70 (or thyroglobulin) and tritiated water, respectively. Even in the presence of high protein/polymer concentrations there was no observable change in either the void volume or the total volume, suggesting the absence of any significant osmotic shrinkage of the gel bed.

3.6. Zonal gel chromatography of dextran sulfate

During dialysis of dextran sulfate solutions at NaCl concentrations equal to or lower than 0.02 M, 50–70% of the macroion passed through the dialysis sac (unpublished observations). So as to ascertain that this effect was not due to degradation, zonal chromatography was performed at various ionic strengths on a Fractogel TSK HW 55 column (0.9 × 60 cm) equilibrated with NaCl (0.005–0.1 M). Sample (0.5 ml, 100 mg/ml) was applied at a flow rate of 18 ml/h, and the column eluate collected in 0.8-ml fractions.

4. Results and discussion

4.1. Partition coefficients for a uniunivalent electrolyte and a macroion

Since extensive partition coefficient data for Na^+ are available from equilibrium dialysis studies of the dextran sulfate-NaCl system [15], that system has also been analyzed by frontal gel chromatography to demonstrate the similarity of the two techniques.

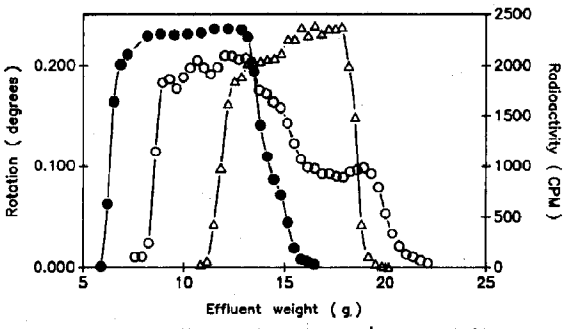

Fig. 2. Elution profiles obtained for Na^+ (○) and Cl^- (△) in frontal gel chromatography of dextran sulfate (35 mg/ml) in 0.1 M NaCl on a column (0.66 × 40 cm) of Fractogel TSK HW 40 equilibrated with 0.1 M NaCl. Notation for plateau regions is in accordance with fig. 1. The elution profile for dextran sulfate (●) was detected by polarimetry. $V_0 = 6.5$ ml and $V_t = 13.2$ ml for this column.

Frontal elution profiles for Na^+ and Cl^- obtained by gel chromatography of dextran sulfate (35 mg/ml) in 0.1 M NaCl show two distinct plateau regions with coefficients of variation ranging between 0.5 and 1.5% (fig. 2). In accordance with fig. 1, the first plateau denotes the total concentration of either Na^+ or Cl^- in the applied mixture $(c_S^\alpha)_m$, whereas the second plateau corresponds to $(c_S^\beta)_m$: the transition between the two plateaux coincides with the disappearance of dextran sulfate from the column, and thus occurs at V_P, as required. Since $\sigma_P = 0$, eq. 10 is the appropriate expression for thermodynamic assessment of the interaction, whereupon the ratio of the two plateau concentrations of S provides a direct measure of the partition coefficient, viz., $(c_S^\alpha)_m/(c_S^\beta)_m$ or $1/R$.

The partition coefficients obtained by frontal gel chromatography are dependent on dextran sulfate concentration and ionic strength as summarized in fig. 3A and B for Na^+ and Cl^-, respectively. The charge concentration of the dextran sulfate derived from the neutrality condition $c^\alpha_{Na^+} = c^\alpha_{Cl^-} + c^\alpha_e$ gave a value of 0.0396 ± 0.0036 ($n = 3$) equiv./l for a 1% dextran sulfate solution (compared with a value of 0.057 that is calculated from the manufacturer's specification of 1.7 sulfate per glycosyl residue).

Partition coefficients obtained by equilibrium dialysis for Na^+ and Cl^- (table 4 of ref. 15) for the dextran sulfate-NaCl systems have also been included in fig. 3. The similarity of results for systems in 0.1 M NaCl clearly attests to the equivalence of equilibrium dialysis and gel chromatography as techniques for obtaining partition coefficients. Although less extensive, results obtained in systems with 0.05 M NaCl are also consistent with the equivalence of the two methods.

Even though no comparable equilibrium dialysis data were available for systems with 0.02 M NaCl due to passage of dextran sulfate through the membrane at low ionic strength (see section 3.6), results of the frontal gel chromatographic technique have been included to show the anomalous behavior of Cl^- partitioning at $I = 0.02$: the

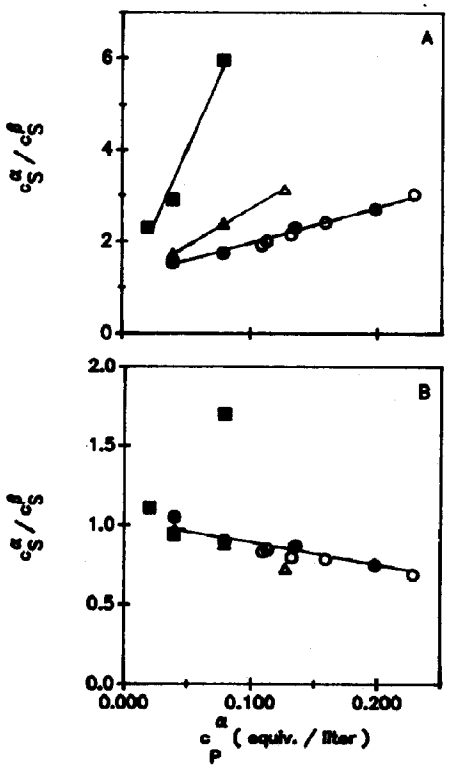

Fig. 3. Comparison of results obtained by frontal gel chromatography (closed symbols) and equilibrium dialysis (open symbols) for the ratio of (A) the Na^+ concentrations and (B) the Cl^- concentrations in the macroion-containing (α) and macroion-free (β) solutions as a function of dextran sulfate concentration in the α-phase. Experiments carried out at 0.1 M NaCl (●, ○), 0.05 M NaCl (▲, △), and 0.02 M NaCl (■). The equilibrium dialysis data are taken from table 4 of ref. 15.

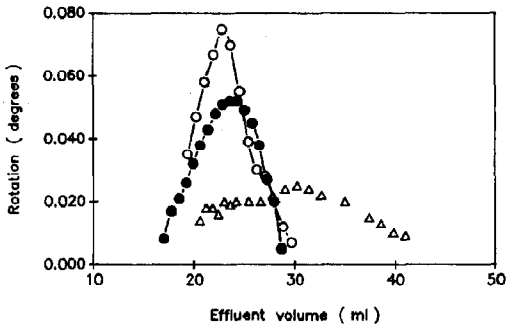

Fig. 4. Elution profiles obtained in zonal gel chromatography of dextran sulfate on a column (0.9×60 cm) of Fractogel TSK HW 55 equilibrated with 0.1 M (○), 0.02 M (●) and 0.005 M (△) NaCl.

value of c_s^α/c_s^β is greater than unity (fig. 3B). The elution profiles for Na^+ exhibited two plateau regions which yielded c_s^α/c_s^β ratios having a linear dependence on the concentration of macroion (fig. 3A), although the β-plateau was less distinct as reflected by higher coefficients of variation (6–10%). This decreased reliability in the magnitude of the β-plateau concentration could be due to (i) a boundary disturbance arising from gravitational instabilities [2–5] at the interface between the polymer solution and the solvent, which would be more evident at low ionic strengths because of the relatively large concentration gradient of diffusible ions; or (ii) the increased trailing of polymer at low ionic strengths as it elutes from the column. In that regard the elution profile obtained in zonal gel chromatography of dextran sulfate is markedly dependent on ionic strength, as shown in fig. 4. In 0.1 M NaCl the dextran sulfate is eluted as a reasonably sharp zone, but the zone is broader when 0.02 M NaCl is used as solvent. In 0.005 M NaCl the dextran sulfate is distributed throughout the profile from V_0 to V_t. This variable gel chromatographic behavior was not due to depolymerization of the dextran sulfate, since readjustment of the low ionic strength sample to 0.1 M NaCl and rechromatography in that medium yielded an identical profile to samples run directly in 0.1 M NaCl (data not shown). However, at low ionic strength ($I = 0.005$) it is likely that electrostatic repulsion between like charges on the dextran sulfate molecule would cause significant extension of the flexible chains [27]. On the basis of fig. 4, it would appear that the presence of 0.02 M NaCl suffices to suppress this effect. The weakly hydrophilic nature of the Fractogel matrix is unlikely to induce nonspecific binding.

Table 1

Gel chromatographic determination of partition coefficients and interaction coefficients for dextran-sorbitol and dextran-NaCl systems

Dextran			Partition coefficient^{-1}	Interaction coefficient (α_{SP})	Covolume (U_{SP}) (l/mol)	
Type	M_n ($\times 10^{-3}$)	Concentration (g/l)	$[R-(c_S^\beta)_m/(c_S^\alpha)_m]$	(l/mol) [a]	Experimental [b]	Predicted
Dextran-sorbitol						
T10	6.0	104	1.115 ± 0.015	6.0 ± 0.7 (3) [c]	9.6	11–18 [d]
T20	16.5	29	1.043	23.6	33.5	30–49 [d]
		71	1.118 ± 0.006	25.6 ± 1.2 (2)	35.5	
T70	39.5	44	1.058 ± 0.001	50.6 ± 0.9 (3)	74.3	71–118 [d]
T200	143	31	1.076 ± 0.022	338 ± 95 (4)	424	256–427 [d]
Dextran-NaCl						
T40	25.4	38.5	1.06	38.4 (Na^+)	53.6	42–76 [e]
			1.06	38.4 (Cl^-)	53.6	

[a] Calculated by application of eq. 9 (dextrans T10 and T20) or eq. 10 to the trailing elution profile from a column of Fractogel TSK HW 40: for the former $\sigma_S = 0.66$, $\sigma_P = 0.085$ (T10) and 0.027 (T20).

[b] Based on the expression $U_{SP} = \alpha_{SP} + M_P\bar{v}_P$ [21] and a value of 0.60 ml/g [20] for the partial specific volume of dextran.

[c] Mean value ± S.D. (figures in parentheses indicate the number of experiments).

[d] Range of values predicted (eq. 11) by the fiber model [20] with the radius of the dextran fiber taken as 0.30 nm [5] and the effective radius of sorbitol as either the Stokes radius of 0.369 nm [29] or 60% thereof [30].

[e] Range of values predicted by the fiber model with the effective radius of electrolyte ions taken as 0.2–0.3 nm.

Preliminary studies, carried out at various flow rates, have indicated that for the dextran sulfate concentrations used in this investigation (up to 5%) there was no dependence of the partition coefficient on flow rate, a result that is consistent with the assumption inherent in eq. 10 that equilibrium is attained in the partitioning of small solute between the gel phase and the mobile phase. Further experiments on the effect of flow rate are currently being conducted at higher concentrations of dextran sulfate.

4.2. Evaluation of thermodynamic interaction coefficients from partition coefficients

4.2.1. Dextran-small solutes

Results obtained from frontal gel chromatographic profiles for dextran-sorbitol and dextran-NaCl mixtures are summarized in table 1. Because the larger dextrans (T40, T70 and T200) were excluded from the gel phase ($\sigma_P = 0$), interaction coefficients (α_{SP}) could be determined by application of eq. 10. On the other hand, the partial inclusion of dextrans T10 and T20 into the gel phase ($\sigma_P = 0.085$ and 0.027, respectively) has led to the use of eq. 9 for calculations of interaction coefficients from data for mixtures containing these polysaccharides as inert macromolecular solute, P. The distribution coefficient obtained for sorbitol (σ_S) was 0.66. Since the interaction coefficients obtained at various sorbitol concentrations were not statistically different, the values reported in table 1 are the means from all experiments with a given dextran. This is in accordance with an implicit assumption inherent in eq. 10.

Before considering the results in detail, it should be noted that any slight error in σ_S and/or σ_P would have no significant effect on the magnitude of the interaction coefficient, since values of α_{SP} obtained by the application of eq. 10 instead of eq. 9 to data for the two smallest dextrans are greater by only 0.3–0.4 l/mol, and thus encompassed by the uncertainty inherent in values determined using the more rigorous expression (eq. 9).

The following points are noted in relation to table 1. (i) The interaction coefficients for the dextran T20-sorbitol system exhibited little, if any, dependence upon polymer concentration, a finding which justifies the neglect of third and higher virial coefficients in the expression for the activity coefficient of small solute. (ii) The α_{SP} obtained by frontal gel chromatography for the dextran T70-sorbitol system (50.6 ± 0.9 l/mol) showed no statistically significant difference from the previously published [5] interaction coefficient (49.1 ± 13.6 l/mol) obtained by equilibrium dialysis. However, for the dextran T200-sorbitol system the present estimate of α_{SP} (338 ± 95 l/mol) is considerably higher than the value of 144 ± 29 l/mol obtained by equilibrium dialysis [5]. (iii) For comparison with predictions based on the excluded volume concept the interaction coefficients are converted to the corresponding covolumes, U_{SP} [21], the inclusion of the $M_P\bar{v}_P$ term in these calculations being a matter of contention [21,28]. (iv) The experimentally determined covolumes increase with increasing size of the dextran, in reasonable quantitative agreement with theoretical prediction. The final column in table 1 presents values of U_{SP} predicted for interaction of a spherical solute (S) and a polymer (P) envisaged as a fiber with radius r_f: from eq. 13 of [20] it is evident that

$$U_{SP} = M_P\bar{v}_P\left[(r_f + r_S)/r_f\right]^2 \qquad (11)$$

where r_f, the radius of the dextran fiber, has been taken as 0.30 nm [5]. A range for each predicted covolume reflects uncertainty about the magnitude of r_S, the effective thermodynamic radius of small solute. For sorbitol systems the larger predicted covolume is calculated using the Stokes radius of 0.369 nm [29] as the estimate of r_S, whereas the lower prediction is based on an effective thermodynamic radius that is 60% of its hydrodynamic counterpart – in keeping with the situation for glycerol and sucrose [30]. The experimental covolumes shown in table 1 lie within the range of the predicted covolumes, not only for systems with sorbitol as S but also for the dextran-NaCl system. (v) The dextran-NaCl system was investigated in order to assess the contribution of the excluded volume effect of the dextran moiety on the NaCl in the previously studied dextran sulfate-NaCl system. We note that the

Table 2

Gel chromatographic determination of partition coefficients and interaction coefficients for globular proteins and PEG-4000 [a]

Protein	Concentration (g/l)	Partition coefficient^{-1} $[R = (c_S^{\beta})_m/(c_S^{\alpha})_m]$	Interaction coefficient (α_{SP}) (l/mol)			
			Experimental [b]		Literature [c]	Calculated [d]
Albumin	10	1.032	208			
	20	1.068	216	216 ± 7	113	312
	30	1.106	222			
Thyroglobulin	10	1.029	1920		220	2300

[a] Conditions: Fractogel TSK HW 40 column equilibrated with 0.03 M Tris-HCl-0.10 M NaCl (pH 7.4).

[b] Calculated by applying eq. 10 to the trailing elution profile for mixtures containing 1 mg/ml PEG-4000.

[c] Values reported by Atha and Ingham [16] on the basis of phase-separation experiments.

[d] Calculated from the expression $\alpha = U_{SP} - M_P\bar{v}_P$ [21], where $U_{SP} = 4\pi N(r_P + r_S)^3/3$ and the radius of PEG-4000 was taken as 1.72 nm [16]. Other parameters were assigned the following magnitudes: albumin – $M_P = 66000$ [31], $\bar{v}_P = 0.734$ ml/g [32] and $r_P = 3.51$ nm [31]; thyroglobulin – $M_P = 670000$, $\bar{v}_P = 0.714$ ml/g and $r_P = 8.61$ nm [25].

interaction coefficient of 38.4 l/mol for the dextran system is consistent with the expected exclusion effects of a flexible dextran chain with $M_n \cong 25000$ on a small solute, but is only 20% of the corresponding value (approx. 200 l/mol) deduced from fig. 3b for the interaction of dextran sulfate with Cl^-. It is therefore concluded that electrostatic effects are the major source of thermodynamic nonideality in the polyelectrolyte-NaCl system.

4.2.2. Globular protein-PEG-4000

Results of gel chromatographic experiments on mixtures with PEG-4000 (1 mg/ml) as small solute and two proteins, bovine serum albumin and thyroglobulin, as P are summarized in table 2, together with the corresponding thermodynamic interaction coefficients. These results again serve to stress the ability of the present procedure to accommodate systems for which the distribution coefficient of small solute is not unity: $\sigma_S = 0.56$ for PEG-4000, whereas $\sigma_P = 0$ for both proteins.

There is a marked disparity between the present magnitudes of α_{SP} and those reported by Atha and Ingham [16] from phase-separation studies. In that regard, calculations of α_{SP} on the basis of spherical geometry for both small solute and protein yield predicted values that are closer to the gel chromatographic estimates of the interaction coefficients than to those based on phase separation. It is possibly relevant that the phase-separation technique used by Atha and Ingham [16] entailed addition of PEG-4000 until the onset of precipitation, conditions that may well favor self-association of protein molecules. Such intra-species interactions would act in opposition to inter-species excluded volume effects and hence lead to lower apparent interaction coefficients for the latter phenomenon.

5. Concluding remarks

The gel chromatographic technique described in the present investigation offers a simple, reproducible procedure for measuring partition coefficients from which thermodynamic interaction coefficients may be determined. These latter parameters quantitatively characterize the thermodynamic nonideality that arises from the interaction of macromolecules with a small solute due to excluded volume and/or electrostatic effects. Its use for the study of ligand binding has, of course, been long recognized [17,33,34]. In measurements of the interaction coefficients, α_{SP}, for dextran-sorbitol, dextran-NaCl and protein-polyethylene glycol systems the coefficient of variation for replicate experiments usually varied between 2 and 12% (the high value observed in experiments with dextran T200 is, however, not understood). These variations are brought about by a 0.2–1.0% change in the ratio of the two plateau concentrations (R) as the result of 0.5–1.5% uncertainty in the estimation of isotopically labelled solutes. Thus, a

minimum difference of 3% in the two plateau concentrations, $(c_B^{\beta})_m$ and $(c_B^{\alpha})_m$, is required to obtain a reliable measure of the interaction coefficient.

Frontal gel chromatography has several advantages over equilibrium dialysis, the procedure used previously to quantify thermodynamic nonideality in terms of interaction coefficients for these polymer-small solute systems [5,15]. Firstly, experiments can be performed and the results analyzed within 2 days, a feature that clearly sets the gel chromatographic procedure apart from equilibrium dialysis. Secondly, the availability of gel chromatographic media with markedly different porosities provides great latitude in the experimental designation of a solute as small. The present method is thus not confined to studies with solutes as small as those considered here, their selection having been dictated by the requirement for comparisons of findings with results obtained by equilibrium dialysis. Even in the event that osmotic shrinkage of the gel beads should occur at high polymer concentrations [35–38], no error would be introduced into the magnitude of the interaction coefficient, α_{SP}, for a small solute with a distribution coefficient, σ_S, of unity [37].

A feature that imparts even further versatility to the gel chromatographic technique is its potential for quantitatively estimating interaction coefficients under conditions where the macromolecular solute is incompletely excluded ($\sigma_P \neq 0$), and/or where accessibility of the gel phase to the small solute is incomplete ($\sigma_S < 1$). From eq. 3 it follows that the only experimental restriction to the determination of α_{SP} by means of eq. 9 or 10 is the requirement that σ_P and σ_S differ sufficiently for the trailing elution profile to exhibit a clearly defined β-plateau region. In situations where $\sigma_P \neq 0$ there is certainly a need to ascertain that the partitioning of the macromolecular solute does not reflect selective access to the gel phase of small members of a polymer population heterogeneous with respect to molecular size. Such a situation may apply to the present results for the interactions of sorbitol with dextrans T10 and T20 (table 1): however, their agreement with theoretical predictions suggests that this effect is minimal. The potential of eq. 9 (or eq. 10) to satisfy gel chromatographic situations in which $\sigma_S \neq 1$ has already been illustrated in the studies with sorbitol ($\sigma_S = 0.66$), NaCl ($\sigma_S = 0.82$) and PEG-4000 ($\sigma_S = 0.56$) as small solute.

The ability to accommodate partial partition of the macromolecule and incomplete partition of the small solute is, in principle, not restricted to the gel chromatographic technique, since eq. 9, with appropriate redefinition of terms, also applies to equilibrium dialysis (a ≡ retentate, b ≡ diffusate). However, the relatively restricted range of membrane porosities available virtually confines the application of equilibrium dialysis to systems comprising a mixture of macromolecular and very small solute species. Frontal gel chromatography should thus be of particular value for the quantitative characterization of thermodynamic nonideality arising from excluded volume effects in concentrated mixtures of macromolecular solutes.

Acknowledgments

This investigation was supported by funds from the Australian Research Grants Scheme.

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