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THE EFFECT OF SOLUTE SIZE AND SHAPE ON THE ELUTION VOLUME IN SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

Comparison is made of the elution, through columns packed with porous glass beads, of polystyrene latex particles in aqueous eluents and linear polystyrenes in tetrahydrofuran. As judged by the elution of species of low molar mass, the characteristics of the packing are the same for both eluents. Provided that allowance is made for the effect of double-layer exclusion on the elution of the latex particles, the weight-average emergence times (ϵ_w) of the particles (spheres) and the polymers (coils) are determined by their hydrodynamic volumes ($[\eta]M$); *i.e.* within experimental error, a common calibration plot of $[\eta]M$ versus ϵ_w can be constructed.

INTRODUCTION

The chromatographic separation of species by size-exclusion chromatography (SEC) is of technological importance and scientific interest. A parameter which has been promoted¹ as the determinant of separation in SEC is the hydrodynamic volume, which is the product of the intrinsic viscosity and the molar mass of the solute ($[\eta]M$). The utility of this "universal" parameter has become accepted for the elution of linear polymers of moderate and high molar mass^{1,2}. However, its applicability to the elution of species of more compact conformation, *e.g.* branched polymers, is less certain²⁻⁴.

We have compared the elution behaviour, through columns packed with Styragel, of linear polystyrenes and polystyrene microgels⁵. Such a comparison is not straightforward since the microgels aggregate in solution and adsorb on the Styragel packing. These effects were avoided⁵ by use of a polar solvent (dimethylformamide) containing electrolyte (lithium bromide). This procedure, however, causes the microgels to be additionally excluded from the pores of the packing to an extent dependent on the thicknesses of the electrical double-layers on packing and microgel. This must be accounted for if meaningful comparison of the elution of linear polystyrene molecules and compact microgels is to be made. When this is done (by extrapolation of elution volumes to infinite electrolyte concentration, hence zero double-layer thickness) the results are in accord with the "universal" parameter concept. This result is particularly striking since the individual contributions to $[\eta]M$ in the two systems

TABLE I
INDIVIDUAL CONTRIBUTIONS TO THE HYDRODYNAMIC VOLUME

	<i>Microgel</i>	<i>Polymer</i>
Elution volume (cm ³)	110	111
Molar mass (10 ⁶ g mol ⁻¹)	55	1.8
Intrinsic viscosity (cm ³ g ⁻¹)	6.1	169
Hydrodynamic volume (10 ⁷ cm ³ mol ⁻¹)	34	30

differ considerably in magnitude as shown by the example⁵ in Table I.

A straightforward theoretical treatment of the exclusion chromatography of microgels is not possible since they are hydrodynamically soft spheres. A more satisfactory comparison would be between the elution of a hard sphere and that of a linear chain. The theoretical prediction based on a simple exclusion mechanism⁶, is that an equivalent elution volume is expected for a coil with hydrodynamic volume $([\eta]M)$ one-half that of a hard sphere.

In our laboratory, polystyrene latex particles (hard spheres) have been characterised by elution through columns packed with porous glass beads by an aqueous eluent containing a low concentration of anionic surfactant and a moderate concentration of electrolyte⁷. When attempts were made to elute polyethylene oxides of varying molar masses through these columns using the same eluent, they were found to adsorb on the glass. Sodium polystyrene sulphonates, which are water-soluble polyelectrolytes, were found to elute at volumes affected by pH and particularly ionic strength. However, a non-aqueous solvent will permit elution of non-electrolytic, non-polar polymers without adsorption and allow access to the same pore volume as that accessible to the aqueous eluent (see below).

In the present work, comparison is made of the elution through columns packed with porous glass beads of polystyrene latex particles (hard spheres) in aqueous eluents with that of linear polystyrenes (coils) in tetrahydrofuran (THF) eluent.

EXPERIMENTAL

Materials

Seven polystyrene latex samples having narrow particle-size distributions were prepared and purified as described earlier⁷. Ten linear polystyrenes having narrow molar-mass distributions were obtained from commercial sources, as indicated in Table II. Average particle diameters (and hence average molar masses, assuming spheres of density 1.06 g cm⁻³) were calculated from transmission electron microscopy measurements made on the latices. For the polymers the molar masses quoted by the manufacturers were generally accepted. An exception was sample P9, for which our own characterisation data (analytical SEC on Styragel with THF eluent and light scattering from solutions in toluene) were preferred. In treating results from SEC we follow the recommendation of Van der Linden⁸ by relating the harmonic mean molar mass, $M_0 = (M_w M_n)^{1/2}$, to the weight-average elution volume. Values of M_0 are given in Table II.

TABLE II

MOLAR MASSES, INTRINSIC VISCOSITIES AND RELATIVE SEC EMERGENCE TIMES FOR LATEX PARTICLES AND LINEAR POLYMERS

Latex particles in aqueous eluent; polymers in THF.

Sample*	M_0^{**} (10^6 g mol^{-1})	$[\eta]^{***}$ ($\text{cm}^3 \text{ g}^{-1}$)	$[\eta]M_0$ ($10^7 \text{ cm}^3 \text{ mol}^{-1}$)	ϵ_w^\S
<i>Polystyrene latex particles</i>				
L1	8.7	***	2.44	0.764
L2	10.3	***	2.88	0.749
L3	73.0	***	19.5	0.690
L4	109	***	30.4	0.670
L5	137	***	30.8	0.664
L6	175	***	48.8	0.645
L7	290	***	81.2	0.619
<i>Linear polystyrenes</i>				
P1	0.050	28.6	0.14	0.873
P2	0.107	48.8	0.52	0.812
P3	0.194	74.0	1.44	0.780
P4	0.37	118	4.38	0.732
P5	0.63	172	10.7	0.692
P6	0.88	215	18.9	0.669
P7	1.64	342	56.2	0.627
P8	1.95	386	75.5	0.628
P9	3.83	634	243	0.579
P10	8.21	966	793	0.544

* Samples obtained from Pressure Chemical Co., Pittsburg, PA, U.S.A. except (i) P8 from Waters Assoc., Milford, MA, U.S.A.; (ii) P9 and P10 from Polymer Laboratories, Church Stretton, Shropshire, U.K.

** M_0 = harmonic mean molar mass ($M_w M_n$)^{1/2}. Values are from ref. 5 (latex particles) or from manufacturer's data (polymers).

*** $[\eta]$ = intrinsic viscosity. Values are $2.8 \pm 0.3 \text{ cm}^3 \text{ g}^{-1}$ (latex particles in aqueous solvent at 25°C) or are calculated from $[\eta] = 0.015 M_w^{0.697}$ (polymers in THF at 25°C; M_w from manufacturer's data). The mean value of $2.8 \text{ cm}^3 \text{ g}^{-1}$ was used for the latex particles in the calculation.

§ ϵ_w = weight-average relative emergence time at 25°C. ϵ is expressed relative to the emergence time of benzyl alcohol, which is the same in both eluents. The values of ϵ_w for the latex particles are obtained by extrapolation to eluent of infinite ionic strength (see text).

Size-exclusion chromatography

Four stainless-steel columns (length $\approx 1 \text{ m}$, internal diameter $\approx 6 \text{ mm}$) were packed, using a dry-packing technique, with glass beads having a wide range of pore diameters. Beads of porosities 7.5–200 nm were from the CPG-10 series, 120–200 mesh (Electro-Nucleonics), whilst the sample of porosity 500 nm was a Fractosil, 130–230 mesh (Merck). The plate count was about 750 m^{-1} , irrespective of eluent. The eluent for the latices was degassed distilled water containing sodium azide (0.1 g dm^{-3} , antifungal agent), Ultrawet-K (commercial-grade sodium dodecylbenzene sulphonate surfactant, 0.4 g dm^{-3} , above the critical micellar concentration), buffering salts (pH ≈ 6 , NaH_2PO_4 and Na_2HPO_4 , maximum concentration $\approx 20 \text{ mmol dm}^{-3}$) and sodium nitrate in sufficient quantity to bring the eluent to the required ionic strength in the range 8–250 mmol dm^{-3} . The eluent for the polystyrene coils was degassed distilled THF. The eluent flow-rate was maintained at $1 \text{ cm}^3 \text{ min}^{-1}$ by

means of a DuPont Instruments Model 870 chromatographic pump. Solutions of samples in the eluent (latices, $c \approx 5 \text{ g dm}^{-3}$; coils, $c \approx 1 \text{ g dm}^{-3}$) were filtered (Millipore, $10 \mu\text{m}$) before being injected into the eluent stream by means of a 2-cm³ loop and a Rheodyne Model 7010 injection valve. Detection of solutes eluting from the columns was by means of a Waters Assoc. Model R4 differential refractometer. The temperature was maintained at $25 \pm 1^\circ\text{C}$ for all experiments.

It is well known that elution volumes are difficult to measure accurately in aqueous SEC⁹. We found it most convenient to express the elution profiles in terms of the emergence-time ratio (ϵ) of the solute relative to that of a low-molar-mass marker, benzyl alcohol. The SEC curves were analyzed by a procedure similar to that described by Pickett *et al.*¹⁰, and the weight-average relative emergence time (ϵ_w) calculated for each sample.

RESULTS AND DISCUSSION

The emergence times of the marker solute were found to be $167 \pm 2 \text{ min}$ with the aqueous eluent, independently of ionic strength, and $167 \pm 1 \text{ min}$ with THF. The agreement between the two emergence times indicates that the pore volume is equally accessible to solutes in both solvent systems*.

As described earlier⁷, the emergence times of the latex samples varied with ionic strength as a result of the variable thicknesses of the electrical double-layers surrounding both solute and packing at finite ionic strength. Values of ϵ_w for the latices unaffected by double-layer effects can be obtained by extrapolation of values obtained at finite values of ionic strength to infinite ionic strength. The procedure⁷ is to plot ϵ_w against $I^{-\frac{1}{2}}$ and extrapolate to $I^{-\frac{1}{2}} = 0$. Values of ϵ_w obtained in this way for the latices are listed in Table II. Values of ϵ_w for the polystyrene coils in THF, also listed in Table II, were obtained directly from the SEC curves.

The intrinsic viscosities of the latices in the eluents have been determined⁷ and found to be $2.8 \pm 0.3 \text{ cm}^3\text{g}^{-1}$, irrespective of ionic strength above 1 mmol dm^{-3} , as expected for electrostatically-stabilised aqueous latices^{12,13}. The near conformity to Einstein's prediction of $[\eta] = 2.5 \text{ cm}^3\text{g}^{-1}$ is indicative of hard spheres. The extra contribution is ascribed to the layer of anionic surfactant adsorbed on the surface of the latex particles. The intrinsic viscosities of the polystyrene coils were calculated from the Mark-Houwink relationship, $[\eta]/(\text{cm}^3\text{g}^{-1}) = 0.015 \bar{M}_w^{0.697}$ for linear polystyrenes in THF at 25°C ¹.

Values of $[\eta]M_0$ are plotted against ϵ_w in Fig. 1. The lines drawn through the points are least squares fits to the results for the 7 latex samples and the 8 polymer samples, P2 to P9. The scatter of results reflects errors of about $\pm 20\%$ in $[\eta]M_0$. Within these errors the results for both species could be fitted satisfactorily with a single line. In any event, the systematic deviation of the results for the polymers from those for the latex particles is very much smaller than Casassa's prediction⁶ (shown by the dashed line in Fig. 1).

From an experimental viewpoint this result reinforces the conclusion reached

* The elution volume (V_e) for a totally permeating species is given by $V_e = V_0 + V_i$, where V_0 is the void volume and V_i is the internal (pore) volume. It is known¹¹ that V_0 of a set of porous glass columns is constant irrespective of the eluent used.

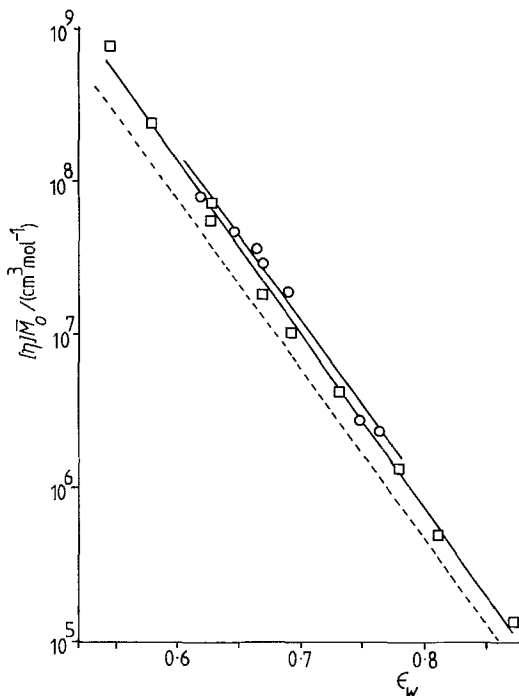


Fig. 1. Semi-logarithmic plot of hydrodynamic volume ($[\eta]M_0$) versus weight-average relative emergence time (ϵ_w) for (○) latex particles and (□) linear polymers. The solid lines are the least squares fits to the results for particles (upper line) and polymers P2 to P9 (lower line). The dashed line is the prediction⁶ for coils relative to the results for hard spheres (*i.e.* latex particles).

earlier⁵ that the separation in SEC depends upon $[\eta]M$ even for compact species, provided that adsorption effects are absent and that the data are treated in such a way that double-layer exclusion effects are circumvented. With regard to the mechanism of separation, the results do not support Casassa's theory of exclusion for coils compared with spheres. Our earlier analysis⁷ of the effect of ionic strength on the elution of latex particles conforms well to a simple exclusion process for spheres; the disagreement between experiment and theory⁶ apparently lies with the coils.

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