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RIGID POLYACRYLAMIDE GELS FOR HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

Cross-linked polyacrylamide microspheres (particle diameter = 10 μm), which are macroporous and rigid, have been examined as a column packing for aqueous high-performance size-exclusion chromatography. Experimental retention data for polysaccharide, poly(ethylene oxide) and poly(ethylene glycol) standards in water suggest that the separation mechanism is size exclusion since a universal calibration plot of log hydrodynamic volume against retention volume is obtained. Columns containing these polyacrylamide gel particles have high efficiencies (around $2 \cdot 10^4$ plates m^{-1}). Experimental plate height data for standards of poly(ethylene glycol) in water demonstrated that the increase in solute dispersion during mass transfer in the polyacrylamide gel particles as the eluent flow velocity is raised is influenced considerably by solute size. These plate height results have been interpreted in terms of an expression permitting the determination of the solute diffusion coefficient in the stationary phase and the polydispersity of the standard. These column packings are suitable for high-resolution separations of oligosaccharides and polysaccharides in water. Peak resolution may be varied by changing eluent flow-rate and temperature.

INTRODUCTION

In a previous paper[1], the preparation of macroporous polyacrylamide microspheres (particle diameter $\approx$ 10 μm) was reported. These cross-linked gel particles are rigid and may be used for separations of water-soluble polymers by high-performance size-exclusion chromatography (HPSEC). Here, we report retention and plate height data for HPSEC separations of poly(ethylene glycol) in water. In view of the use of the plot of log hydrodynamic volume against retention volume V_R for universal calibration of polymers[2], an important objective in this work was to examine this plot for aqueous separations with these cross-linked polyacrylamide packings. A second objective was to interpret the dependence of plate height on eluent flow-rate with the treatment previously studied for silica-based packings[3,4], in order to

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evaluate the diffusion coefficient of poly(ethylene glycol) in cross-linked polyacrylamide gel and to determine the polydispersity of each poly(ethylene glycol) standard. A third aim was to demonstrate high-resolution separations of polysaccharides with these crosslinked gel particles.

EXPERIMENTAL

Macroporous polyacrylamide gel particles were produced in an inverse suspension process by co-polymerizing acrylamide and N,N′-methylenebisacrylamide with the cross-linking agent as the major monomeric component. The inverse suspension polymerizations were performed according to the conditions described elsewhere[5]. The cross-linked polyacrylamide particles were separated by air classification, and optical and scanning electron micrographs indicated a narrow particle size distribution from which the mean particle diameter was determined. The dry particles were dispersed in methanol, and this slurry was packed into a column (300 × 7 mm I.D.) at pressures up to 3500 p.s.i. (1 p.s.i. ≡ 6894.8 N m^{-2}). Such columns having the name PLaquagel are produced by Polymer Labs., Church Stretton, U.K., and columns designated J56 and J70 were used for the universal calibration and column efficiency experiments. Column NP1 (200 × 7 mm I.D.) was employed for the high-resolution separations.

The chromatographic apparatus consisted of a Knauer pump Model 64 (Knauer, Berlin, F.R.G.), a Rheodyne Model 7125 injection valve (100-μl loop), supplied by HPLC Technology, Macclesfield, U.K., and a Knauer differential refractometer No. 98. Solute concentrations were typically 0.1% (w/v). It was established that no change in V_R occurred for more dilute solutions. Water (doubly distilled from glass, degassed and filtered) containing sodium azide (0.1 g l^{-1}) was used as the eluent in the SEC separations. The polymeric solutes were poly(ethylene glycol), poly(ethylene oxide) and polysaccharide standards (Polymer Labs.), which were designated PEG, PEO and PSA respectively followed by a number corresponding to the molar mass (g mol^{-1}). The other solutes were pentadecaethylene glycol[6,7] (PDG having molar mass = 678 g mol^{-1}) kindly provided by Dr. C. Booth, University of Manchester, Manchester, U.K., Frodex 42 (a corn syrup polysaccharide) kindly provided by Dr. T. Tweeten, Hewlett-Packard, Avondale, PA, U.S.A., and absolute ethanol, glucose, sucrose and raffinose supplied by Fisons Scientific Equipment Division, Loughborough, U.K. Retention data were expressed either in time (min) or in terms of a percentage of the retention volume V_T for the totally permeating solute ethanol which was used as an internal standard in all injected solutions. Molar mass calibration curves were employed to establish the interstitial (or void) volume V_0 of a column and the linear flow velocity of the eluent was calculated with the use of V_0. This value of V_0 was also employed in the calculation of the retention ratio R of each solute, defined by $R = V_0/V_R$. Values of plate number (N) and plate height (H) were calculated from experimental chromatograms by the width-at-half-height method.

The solution viscosity of poly(ethylene glycol) and polysaccharide standards in distilled water was measured with an Ubbelohde suspended level dilution viscometer at 298 K. The flow time for distilled water was 279 s, and kinetic energy corrections were therefore not significant. The viscometer allowed up to 5 successive dilutions, and the initial concentration of polymer in the viscometer was adjusted so that

the relative viscosity was below 2 and so that the most dilute solution had a relative viscosity above 1.2. Solution viscosity data were extrapolated linearly by Huggins and Kraemer plots to infinite dilution in order to find the intrinsic viscosity $[\eta]$ (dl g^{-1}).

RESULTS AND DISCUSSION

Universal calibration

Plots of molecular weight or molar mass M (g mol^{-1}) against V_R for PSA, PEG and PEO standards in water are shown in Fig. 1. The PSA, PEG and PEO standards all have low polydispersity ($\bar{M}_w/\bar{M}_n < 1.1$), where $\bar{M}_w$ and $\bar{M}_n$ are the weight average and number average molar masses respectively, and so the placement of the calibration curve using V_R at the peak height maximum of a chromatogram will therefore be accurate[2]. The experimental results in Fig. 1 show that the M_{PSA} calibration curve is displaced to high V_R compared with the curve for the PEG and PEO standards, suggesting that at a given value of M a PSA standard has a smaller size in solution than PEG.

At a given value of V_R assuming the hydrodynamic volume of a solute to be the size parameter for universal calibration, we can write

$$\log [\eta]_P M_P = \log [\eta]_{PSA} M_{PSA} \quad (1)$$

where the subscript P refers to a calibration established experimentally with standards

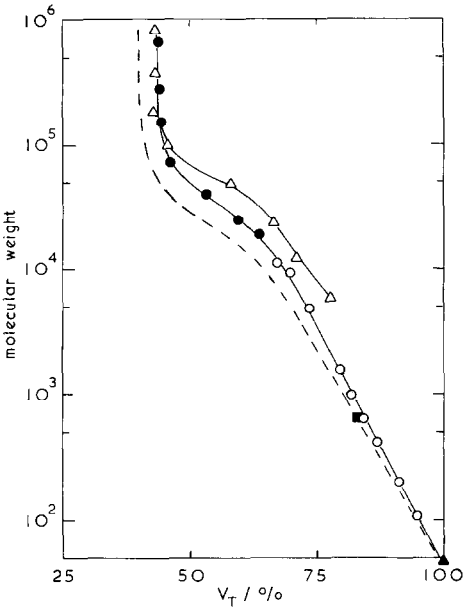


Fig. 1. Molecular weight calibration curves for water as eluent (flow-rate 1 cm^3 min^{-1}) at room temperature. ——, Column J56 (length 30 cm) with (△) polysaccharide standards, (○) poly(ethylene glycol) standards, (●) poly(ethylene oxide) standards, (▲) ethanol; – – –, column J70 (length 30 cm) with poly(ethylene glycol) and poly(ethylene oxide) standards and (■) PDEG.

TABLE I

INTRINSIC VISCOSITIES OF POLYMER STANDARDS IN WATER AT 298 K

Standard	$[\eta]$ (dl g^{-1})
PEG4820	0.129
PEG9200	0.191
PEO18000	0.369
PEO39000	0.589
PEO86000	0.923
PSA5800	0.084
PSA12200	0.127
PSA23700	0.170
PSA48000	0.254
PSA100000	0.416

of PEG and PEO. Eqn. 1 assumes that column, solvent and temperature remain constant. A test of eqn. 1 for the standards listed in Table I whose retention behaviour for column J56 is displayed in Fig. 1 is shown in Fig. 2. For values of $[\eta]M$ above $2 \cdot 10^3$ dl mol^{-1}, hydrodynamic volume may be regarded as a reasonable representation of separation behaviour suggesting that separations of these polymers are dominated by a size-exclusion mechanism. At low hydrodynamic volumes corresponding to molar masses below 10^4 g mol^{-1}, the PSA and PEG curves clearly diverge. Similar behaviour has been observed for short polycarbonate and polystyrene chains in chloroform[8], and two possible explanations were proposed which may apply to the behaviour of low polymers in Fig. 2. First, short PSA chains having a bulky repeating unit may adopt a different chain conformation from that of PEG, and so short PSA and PEG chains do not display the same hydrodynamic behaviour. Second, solute-gel interactions for small molecules have been observed to generate divergent calibrations[9].

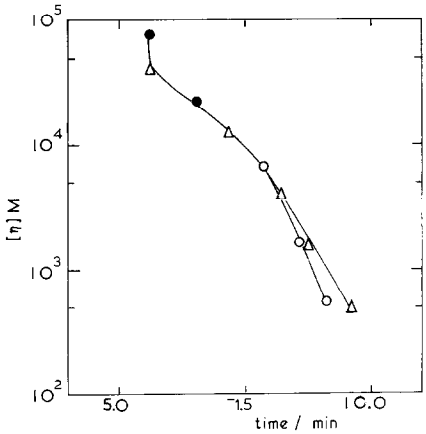


Fig. 2. Hydrodynamic volume calibration curves for column J56 with water as eluent (flow-rate 1 cm^3 min^{-1}) at room temperature. (△) Polysaccharide standards, (○) poly(ethylene glycol) standards, (●) poly(ethylene oxide) standards.

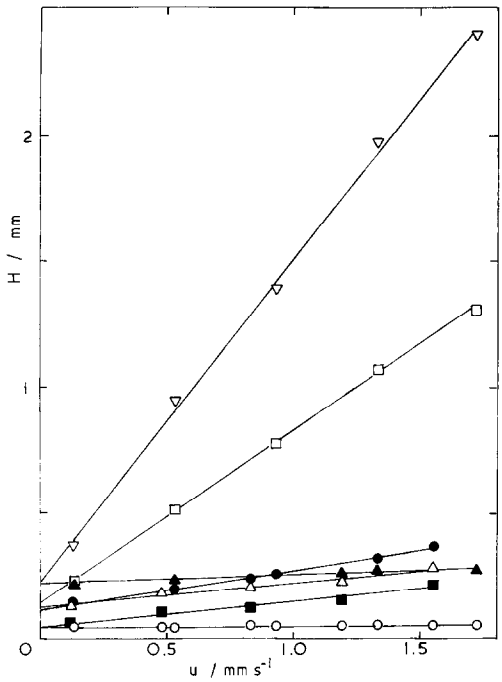


Fig. 3. Dependence of experimental plate height on eluent flow velocity for poly(ethylene glycol) standards in water at room temperature with column J70 (▽) PEG9200; (□) PEG4820; (●) PEG1000; (■) PDEG; (△) PEG630; (▲) PEG200; (○) ethanol.

Column efficiency

Values of N for ethanol were typically around $2 \cdot 10^4$ plates m^{-1} for columns packed with particles having a diameter $d_p = 10$ μm. Curves for J70 showing the dependence of H for samples of PEG, PDEG and ethanol on the linear flow velocity u of the eluent are shown in Fig. 3. The plot for each solute in Fig. 3 exhibits reasonable linear behaviour, and so data for H may be interpreted in terms of the relation

$$H = 2\lambda d_p + [R(1 - R)\, u d_p^2/30 D_s] + (L \ln [\overline{M}_w/\overline{M}_n]_T/D_2^2 V_R^2) \quad (2)$$

in which λ (close to unity) is a constant characteristic of the packing, D_s is the diffusion coefficient of the solute in the stationary phase, L is the column length, $[\bar{M}_w/\bar{M}_n]_T$ is the polydispersity of the solute, and D_2 is the slope of the SEC calibration relation between ln molar mass and V_R. The first two terms in eqn. 2 arise from eddy diffusion due to solute dispersion in the mobile phase and from solute dispersion owing to mass transfer in the stationary phase respectively, as in liquid chromatography. The polydispersity term has been derived previously[10]. Methods have been discussed previously for evaluating with eqn. 2 values of D_s and $[\bar{M}_w/\bar{M}_n]_T$ from chromatograms for polystyrene standards and proteins obtained in HPSEC separations with silica-based column packings[3,4].

TABLE II

DIFFUSION COEFFICIENTS OF POLY(ETHYLENE GLYCOL) STANDARDS

Standard	$D_m/10^{-7}$ ($cm^2\ s^{-1}$)	$D_s/10^{-8}$ ($cm^2\ s^{-1}$)	D_s/D_m
PEG200	39.0	21.30	0.055
PEG630	31.4	8.91	0.028
PDEG	30.8	7.82	0.025
PEG1000	26.5	5.17	0.020
PEG4820	11.9	1.19	0.010
PEG9200	10.9	0.63	0.006

For PEG standards in Fig. 3, it is apparent that the slope of each curve exhibits an increase as the molar mass of PEG is raised. In terms of eqn. 2, the explanation for this dependence of slope on M is the decrease in diffusion coefficient for longer chains (which will have higher mass transfer dispersion). Results for D_s for PEG standards calculated from Fig. 3 are given in Table II. The calculation with eqn. 2 involved the determination of the slope D_2 for each solute from the calibration curve for column J70 in Fig. 1. In Table II we show values for the diffusion coefficient for a solute in the mobile phase D_m which is assumed to be the diffusion coefficient of PEG (or PEO) in free solution at infinite dilution. Values for D_m were taken from tabulated data given elsewhere[11]. The derived data for D_s in Table II are much less than values of D_m. Whilst errors in the procedure for determining D_s may result from the choice of value for d_p, since d_p^2 appears in the second term in eqn. 2, our crosslinked polyacrylamide microspheres do have narrow particle size distributions (see ref. 1). The results in Table II suggest that D_s/D_m increases as the molar mass of the solute is reduced, in agreement with earlier experimental work as discussed previously[3,4]. Studies of polystyrene standards (M = 3600–35000 g mol^{-1}) separating in tetrahydrofuran with silica-based packings (d_p = 8 μm) suggested that D_s/D_m was in the range 0.07–0.14. The lower values of D_s/D_m for PEG4820 and PEG9200 indicate that these solutes in water are subjected to restricted diffusion in SEC with crosslinked polyacrylamide gels at ambient temperature. From the calculations performed previously[3,4], it is expected that the mobile phase dispersion of PEG solutes having a value of D_m around 10^{-6} $cm^2\ s^{-1}$ will be dominated by eddy diffusion because mobile phase dispersion mechanisms which depend on u, *e.g.* longitudinal molecular diffusion and mass transfer, may be neglected for polymeric solutes having low values of D_m with u in the range 1–3 mm s^{-1}. Consequently, it appears that restricted diffusion of solutes during mass transfer into the stationary phase is higher for crosslinked polyacrylamide microspheres than for silica-based packings.

From the data in Fig. 3 values of $[\bar{M}_w/\bar{M}_n]_T$ may be evaluated with eqn. 2 provided the first term for mobile phase dispersion is known accurately. Two methods have been considered previously[3,4]. First, we may assume λ = 1.0 when the eddy diffusion contribution to H will be 20 μm. Second, we may assume that the mobile phase dispersion of a non-permeating polymer is close to the value of H for ethanol which is not polydisperse, so that the eddy diffusion contribution will be 54 μm corresponding to the average value of H for ethanol for u in the range 0.1–1.8 mm s^{-1}. A third method is available involving the monodisperse solute PDEG. Extra-

TABLE III

POLYDISPERSITIES OF POLY(ETHYLENE GLYCOL) STANDARDS

Standard	$\bar{M}_w/\bar{M}_n$*	$[\bar{M}_w/\bar{M}_n]_T$**	$[\bar{M}_w/\bar{M}_n]_T$***	$[\bar{M}_w/\bar{M}_n]_T$§
PEG630	1.080	1.041	1.061	1.048
PEG1000	1.060	1.029	1.047	1.035
PEG4820	1.040	1.038	1.050	1.041
PEG9200	1.080	1.033	1.038	1.034

* SEC characterization with cross-linked polystyrene gels.

** Determined with eqn. 2 using eddy diffusion term given by plate height for ethanol.

*** Determined with eqn. 2 using $\lambda = 1$ in eddy diffusion term.

§ Determined with eqn. 2 using eddy diffusion term given by plate height for PDEG extrapolated to $u = 0$.

polation of the data for PDEG in Fig. 3 to $u = 0$ will give an intercept which arises solely from dispersion due to eddy diffusion, and it is evident in Fig. 3 that this intercept is very close to values of H for ethanol. Values of $[\bar{M}_w/\bar{M}_n]_T$ are given in Table III, where reasonable agreement with polydispersity results obtained by SEC characterization[12] of PEG standards in tetrahydrofuran with cross-linked polystyrene gels is observed.

High-resolution separations

The calibration curves in Fig. 1 indicate that the cross-linked polyacrylamide microspheres have high pore volume. Furthermore, calibrations for solutes having molar masses between 40 and 10^4 g mol^{-1} exhibit good linearity. We have shown

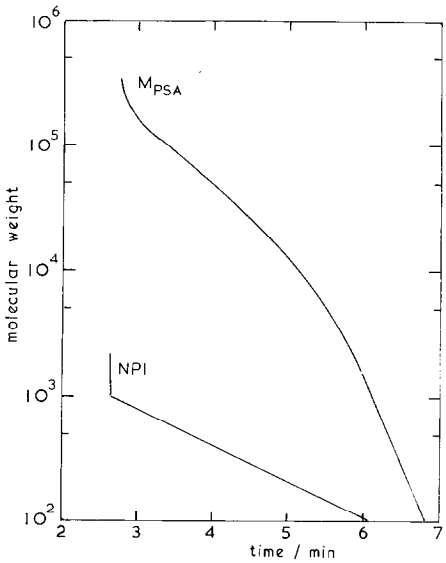
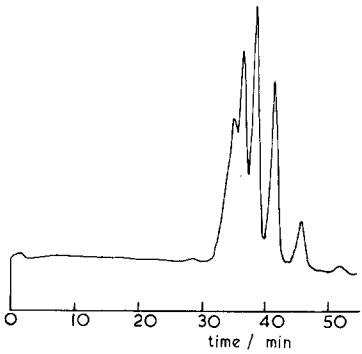


Fig. 4. Molecular weight calibration curve for polysaccharide standards in water as eluent (flow-rate 1 cm^3 min^{-1}) at room temperature with column NP1 (length 20 cm). Curve M_{PSA} calculated from Fig. 1 for column with length = 20 cm.



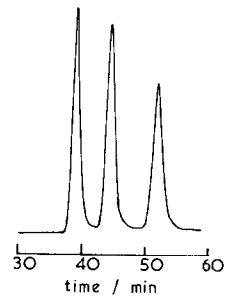
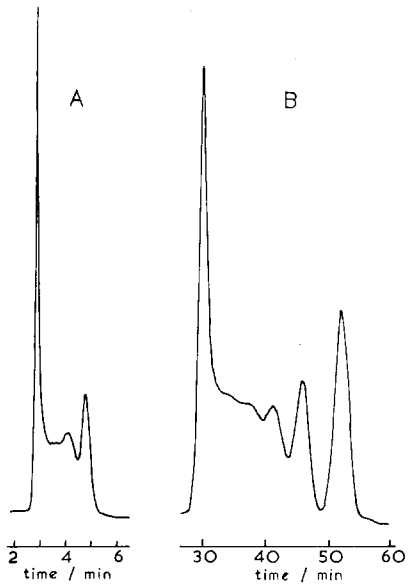


Fig. 5. High-resolution separation of PEG200 standard in water as eluent (flow-rate 0.1 $cm^3\ min^{-1}$) at room temperature with column NP1 (length 20 cm).

Fig. 6. High-resolution separation of a mixture of glucose, sucrose and raffinose in water as eluent (flow-rate 0.1 $cm^3\ min^{-1}$) at room temperature with column NP1 (length 20 cm).

previously the potential of this column packing for high-resolution separations by examining a cocktail of PEG standards[1]. Column packing NP1 was produced with small pore sizes with the aim of performing high-resolution separations of low polymers, prepolymers and small molecules in aqueous media. The calibration curve for column NP1 is shown in Fig. 4, demonstrating high pore volume, and the NP1 particles have sufficient rigidity for use in HPSEC columns at a volumetric flow-rate of 1 $cm^3\ min^{-1}$.



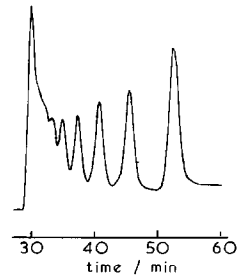


Fig. 7. High-resolution separations of a Frodex 42 corn syrup in water as eluent at room temperature with column NP1 (length 20 cm). (A) Eluent flow-rate 1 $cm^3\ min^{-1}$; (B) eluent flow-rate 0.1 $cm^3\ min^{-1}$.

Fig. 8. High-resolution separation of a Frodex 42 corn syrup in water as eluent (flow-rate 0.1 $cm^3\ min^{-1}$) at 353 K with column NP1 (length 20 cm).

High-resolution separations with column NP1 may be demonstrated at a volumetric flow-rate of 0.1 cm^3 min^{-1}, as shown in Figs. 5–7. The resolution of the components in the PEG200 standard shown in Fig. 5 was not possible in the study performed to obtain the data in Fig. 3 where a single peak was observed. The calibration curve in Fig. 4 and the resolution displayed in Fig. 6 clearly suggest that the column NP1 is capable of providing excellent separations of oligosaccharides having low molar mass. The chromatographic profile obtained by HPSEC with this NP1 packing is capable of distinguishing various polysaccharide corn syrups. An example of one such profile is shown in Fig. 7, demonstrating how resolution is markedly affected by eluent flow-rate working at low eluent flow-rate (higher column efficiencies) and by high temperature (lower eluent viscosity and higher solute diffusion coefficients) as shown in Fig. 8.

CONCLUSIONS

Our studies of standards of polysaccharides, poly(ethylene glycol) and poly(ethylene oxide) in water demonstrate that separations with cross-linked polyacrylamide gels operate by a size-exclusion mechanism, since universal calibration in terms of hydrodynamic volume is observed. These column packings have high efficiencies for high-performance separations. The contribution to plate height from mass transfer in the stationary phase, which is considerable at fast eluent velocities for standards having molar masses around 10^4 g mol^{-1}, has been evaluated from experimental plate height data. Calculation of the diffusion coefficient of poly(ethylene glycol) in the stationary phase indicates that polymer diffusion in the macroporous polyacrylamide packing is restricted. The contribution to experimental plate height arising from the polydispersity of poly(ethylene glycol) has been estimated. High-resolution separations of oligosaccharides and polysaccharides may be obtained with these rigid polyacrylamide microspheres.

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