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HIGH-PERFORMANCE GEL PERMEATION CHROMATOGRAPHY WITH SILICA MICROSPHERES: MASS-TRANSFER DISPERSION AND POLYDISPERSITY OF POLYSTYRENE

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SUMMARY

Separations of polystyrene standards by gel permeation chromatography have been performed with columns containing silica microspheres having a particle diameter of 8 μm . Experimental plate-height data for polystyrene have been interpreted in terms of an expression in which the only mobile-phase dispersion term results from eddy diffusion. This simplified expression permits the evaluation of the contribution to chromatogram broadening from solute mass transfer in the stationary phase. Values of the diffusion coefficient of polystyrene in the stationary phase have been calculated from the mass-transfer contribution, suggesting that restricted diffusion occurs. A procedure is proposed for evaluating the mobile-phase dispersion term, which then permits the determination of the polydispersity for polystyrene from experimental plate-height data.

INTRODUCTION

In a previous paper¹, we reported experimental plate-height data for permeating polystyrene standards in gel permeation chromatography (GPC) with columns containing silica microspheres (particle diameter $\approx 20 \mu\text{m}$). The contributions to chromatogram broadening arising from solute-dispersion mechanisms in the mobile phase and during mass transfer in the stationary phase were assessed as a function of the eluent flow velocity (u) and the diffusion coefficient of the solute. Data for the plate height (H) for a solute having a constant retention volume (V_R) over the range of u with a column of length L were interpreted in terms of the expression

$$H = A + (B/u) + Cu + (\sigma_M^2/L) \quad (1)$$

in which A , B and C are coefficients depending on several parameters (see later), where the first term (A) is the eddy diffusion term for solute dispersion in the mobile phase,

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the second term (B/u) results from dispersion owing to molecular diffusion in the longitudinal direction in the mobile phase, the third term (Cu) results from solute dispersion owing to mass transfer in the stationary phase, and the fourth term contains the standard deviation σ_M for the true molecular weight distribution, which may be related to the true polydispersity defined as the ratio of the weight-average and number-average molecular weights $[\overline{M}_w/\overline{M}_n]_T$. The first three terms follow from the equation derived by Van Deemter *et al.*², and the polydispersity term is added as proposed by Knox and McLennan³.

Our experimental results¹ indicated that non-permeating polystyrenes exhibited very little or no change in H as u was varied from 0.03 to 1.1 cm sec⁻¹. Consequently, the second term in eqn. 1 may be omitted, and the expression for H with the coefficients A and C written out in full is given by

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

where λ (close to unity) is a constant characteristic of the packing, d_p is the particle diameter of the column packing, R is the retention ratio, defined here for each solute by V_0/V_R , where V_0 is the interstitial (or void) volume of the column, D_s is the diffusion coefficient of the solute in the stationary phase, and D_2 is the slope of the GPC calibration relation between \ln molecular weight and V_R . The first two terms in eqn. 2 follow from the theoretical treatment of Giddings⁴ and the polydispersity term has been derived previously¹.

In this paper, we report experimental plate-height data for permeating polystyrene standards in GPC with columns containing silica microspheres having $d_p \approx 8 \mu\text{m}$. The true polydispersity of polystyrene standards is determined from the experimental dependence of H on u with the aid of eqn. 2. This necessitates evaluation of the contribution of plate height arising from mass-transfer dispersion in the stationary phase, from which values of D_s have been estimated. The procedure for determination of polydispersity is more precise than the approximate method proposed earlier¹, which involved the use of plate-height data at low u when the much reduced contribution from mass-transfer dispersion was neglected.

EXPERIMENTAL

The sample of SG30 silica was kindly provided by Dr. J. D. F. Ramsay and Dr. D. C. Sammon of AERE (Harwell, Great Britain). Examination of the silica microspheres by scanning electron microscopy and with a Coulter counter suggested that the mean particle diameter was about $8 \mu\text{m}$. A column (25 cm \times 0.8 cm I.D.) was packed by pumping a methanol slurry of SG30 silica at $5.86 \cdot 10^6 \text{ N m}^{-2}$ according to the method of Bristow *et al.*⁵. At the top of the column, the silica packing was covered by a stainless-steel mesh above which were placed ballotini, which, in turn, were covered by a porous PTFE plug. A syringe-injection head was then attached at the top of the column.

GPC separations were performed with a Perkin-Elmer Model 1220 positive-displacement syringe pump operated as described elsewhere¹. Detection of solutes was performed with an ARL ultraviolet detector (254 nm; cell volume $8 \mu\text{l}$; air as reference). The eluent was tetrahydrofuran (BDH, Poole, Great Britain), which was

destabilized, stored over calcium hydride for 4 h, and then distilled from calcium hydride and copper(I) chloride. The solutes were AnalaR toluene (BDH), tetraphenylethylene (TPE) (Aldrich, Milwaukee, WI, U.S.A.) and polystyrene standards (Waters Assoc., Milford, MA, U.S.A.), which are designated PS followed by a number corresponding to the molecular weight. The calibration curve for the column of SG30 silica was established at an eluent flow-rate of $1 \text{ cm}^3 \text{ min}^{-1}$ with injections ($10 \mu\text{l}$) of polystyrene standards ($\leq 0.2\%$, w/v), TPE (0.01% , w/v), and toluene (0.2% , w/v) in tetrahydrofuran. Column efficiencies at varying flow-rates were established with a solute concentration of 0.2% (w/v) in an injection volume of $10 \mu\text{l}$ (except for TPE, which had a concentration of 0.01% , w/v). The plate height was calculated from an experimental chromatogram by the width-at-half-height method.

RESULTS

The GPC calibration curve for SG30 silica is shown in Fig. 1. The shape of this curve suggests that the pore-size distribution is almost identical to that of the H4 silica examined previously¹. From the curve in Fig. 1, the value of V_0 is estimated to be 4.98 cm^3 , which was used in calculation of R for each polystyrene standard.

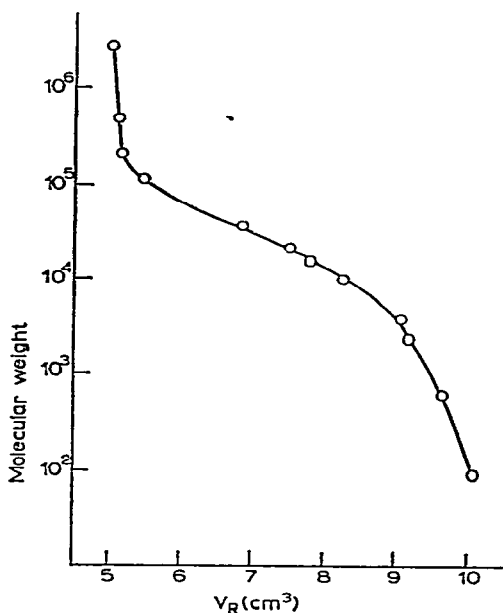


Fig. 1. GPC calibration curve for polystyrene standards, TPE and toluene with SG30 silica.

Curves showing the dependence of H on eluent flow-rate for SG30 silica are given in Fig. 2; the linear velocity, u , was calculated with use of V_0 . It is evident from Fig. 2 that the dispersion term for longitudinal molecular diffusion in eqn. 1 is important for small molecules, as minima are observed in plots of H against u for toluene, TPE, and polystyrene standard PS-600; no minima are observed for polystyrene

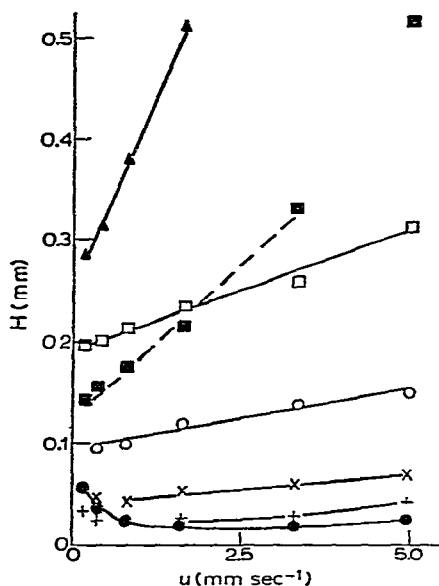


Fig. 2. Dependence of experimental plate height on eluent flow velocity for polystyrene standards eluted from SG30 silica: \blacktriangle , PS-110000; \blacksquare , PS-35000; \square , PS-9800; \circ , PS-3600; \times , PS-600; $+$, TPE; \bullet , toluene.

standards of higher molecular weight. Consequently, it is assumed that dispersion due to longitudinal molecular diffusion is unimportant for high polymers at $u > 1 \text{ mm sec}^{-1}$, and this dispersion term is not included in eqn. 2.

For polystyrene standards PS-3600, PS-9800, PS-35000 and PS-110000 in Fig. 2, it is apparent that the slope of each curve exhibits an increase as the molecular weight of the polystyrene is raised. The divergence of these curves as u increases may be interpreted in terms of the dispersion term in eqn. 2 for mass transfer in the stationary phase. Values of the diffusion coefficient of polystyrene in the mobile phase D_m , assumed to be the diffusion coefficient of a molecule in free solution at infinite dilution, have been calculated as described elsewhere⁶ and are given in Table I. Therefore, the explanation for the molecular-weight dependence of the slopes of the curves in Fig. 2 is the decrease in the diffusion coefficient for larger molecules (which will have higher mass-transfer dispersion). Eqn. 2 was used to evaluate D_s for polystyrene standards from the slopes of the plots in Fig. 2. The data are shown in Table I, assuming $d_p = 8 \mu\text{m}$. The choice of particle diameter is critical, since d_p^2 appears in eqn. 2. However, our silica microspheres have narrow particle size distributions (see ref. 1). Be-

TABLE I

DIFFUSION COEFFICIENTS OF POLYSTYRENE STANDARDS

Polystyrene	$D_m/10^{-7} (\text{cm}^2 \text{sec}^{-1})$	R	$D_s/10^{-8} (\text{cm}^2 \text{sec}^{-1})$	D_s/D_m
PS-3600	28.9	0.55	41.6	0.144
PS-9800	15.8	0.60	20.7	0.131
PS-35000	7.4	0.73	6.0	0.082

cause the plot of H against u for PS-35000 is a curve in Fig. 2, the result at $u = 5 \text{ mm sec}^{-1}$ was omitted in the determination of the slope. Data for PS-110000 have not been calculated because this polystyrene standard has V_R close to the exclusion limit (see Fig. 1), so that misshaped peaks, particularly at high u , may occur because part of the solute is excluded while the remainder permeates some pores. The derived data for D_s in Table I are much less than the values of D_m . Knox and McLennan⁷ have determined results for D_s by evaluating mass-transfer dispersion for polystyrene standards separating with a similar porous silica ($d_p \approx 7.5 \mu\text{m}$). Their results show that D_s/D_m in the range 0.059–0.167 increases as the molecular weight of the polystyrene is reduced from 33,000 to 2000. Although our calculation procedure is simpler than that used by Knox and McLennan⁷, our results for D_s/D_m in Table I are in good agreement. Other estimates of D_s have been obtained with larger particles. Giddings *et al.*⁸ studied chromatogram broadening with porous glass particles having diameters in the range 44–74 μm and found that D_s was about $D_m/6$ for polystyrenes with molecular weights <5000. Van Kreveld and Van den Hoed⁹ evaluated the dispersion term for mass transfer within porous silica particles ($d_p = 75\text{--}125 \mu\text{m}$), finding D_s/D_m to decrease from 0.31 to 0.12 as the molecular weight of polystyrene standards increased from 20000 to 160000. Somewhat higher values of D_s/D_m of 0.19 (PS-9800) and 0.11 (PS-35000) than those given in Table I were determined from the dependence of H on u for the H4 silica (see ref. 1).

Values of $[\overline{M}_w/\overline{M}_n]_T$ may be evaluated from Fig. 2, provided that the first term for mobile-phase dispersion in eqn. 2 is known. It was observed previously that the experimental curves for the dependence of H on u for toluene and non-permeating polystyrene standards were almost coincident, the value of H for PS-1987000 exhibiting little change as u was varied¹. Consequently, it was proposed that plate-height data for toluene H_t can represent the first term in eqn. 2 for a high polymer. Since the mass-transfer term may be evaluated from the slope of the curve for each polystyrene in Fig. 2, values of $[\overline{M}_w/\overline{M}_n]_T$ may be determined with eqn. 2 and are given in Table II. The value of $[\overline{M}_w/\overline{M}_n]_T$ for PS-35000 at $u = 5.02 \text{ mm sec}^{-1}$ is expected to be overestimated because the value of H for this polystyrene standard was not included in the determination of the slope of the curve of H against u for the calculation of D_s from the dispersion term for mass transfer in the stationary phase. Apart from PS-3600, the data for polydispersity are reasonable, being somewhat higher than the theoretical values of 1.011 (PS-9800) and 1.003 (PS-35000) calculated for polystyrenes prepared by a "living" anionic polymerisation¹⁰. Somewhat higher values will be expected in practice because of the rigorous conditions required in the experimental

TABLE II

POLYDISPERSITIES OF POLYSTYRENE STANDARDS OBTAINED FROM PLATE-HEIGHT DATA AT VARIOUS ELUENT FLOW-RATES

Polystyrene	$[\overline{M}_w/\overline{M}_n]_T$		
	$u = 1.67 \text{ mm sec}^{-1}$	$u = 3.35 \text{ mm sec}^{-1}$	$u = 5.02 \text{ mm sec}^{-1}$
PS-3600	1.017	1.017	1.013
PS-9800	1.033	1.029	1.031
PS-35000	1.010	1.010	1.018

polymerisation technique. The theoretical value of $[\overline{M}_w/\overline{M}_n]_T$ for PS-3600 is 1.029, indicating that the values in Table II are too low. This may arise because the calibration curve in Fig. 1 suggests that PS-3600 is separating close to the total permeation volume. We have taken a constant value of D_2 , assuming a linear plateau region of the calibration curve when calculating $[\overline{M}_w/\overline{M}_n]_T$ with eqn. 2, although in practice the slope of the calibration curve will change as the total permeation volume is approached. The low values of $[\overline{M}_w/\overline{M}_n]_T$ may also result from incomplete fractionation of chains in a polymer eluting close to the total permeation volume. The data in Table II suggest that the permeating polystyrene standards have narrow distributions.

DISCUSSION

In the first detailed theoretical treatment of chromatogram broadening in GPC, Giddings and Mallik¹¹ proposed an expression for H which, for a monodisperse solute, we shall write in the form

$$H = (B/u) + (Cu) + \Sigma 1/[(1/A) + (1/C_m u)] \quad (3)$$

in which the first two terms correspond to the dispersion terms defined in eqn. 1. The third term in eqn. 3 is a mobile phase dispersion term containing contributions from eddy diffusion (A) and mass transfer ($C_m u$). This term in the treatment of Giddings and Mallik¹¹ may be written in the form

$$1/[(1/2\lambda d_p) + (D_m/w_t d_p^2 u)] \quad (4)$$

where w_t is regarded as a geometrical factor that may be assumed to be about unity. For a polymer with a molecular weight of *ca.* 20,000 having a value of D_m around 10^{-6} cm² sec⁻¹ (see ref. 6), the second term in the denominator in relation 4 at $u = 2.5$ mm sec⁻¹ and with $d_p = 8$ μ m will be about 1% of the first term in the denominator assuming $\lambda = 1.0$. We may conclude that, for high polymers, the eddy-diffusion term dominates mobile-phase dispersion, and there is abundant experimental evidence that non-permeating macromolecules exhibit little or no change in H as u is raised^{1,6}. Further the mobile-phase dispersion term (B/u) may be neglected for high polymers when $u > 0.3$ mm sec⁻¹ (see ref. 1), so, for a monodisperse high polymer, eqn. 3 simplifies to the dispersion terms given in eqn. 2.

Eqn. 3 permits us to evaluate H for toluene theoretically. With the expressions for A , B , C and C_m proposed by Giddings and Mallik¹¹, we find that $H_t = 16.3$ μ m at $u = 2.5$ mm sec⁻¹ and $H_t = 17.35$ μ m at $u = 5.0$ mm sec⁻¹ with $d_p = 8$ μ m, $D_m = 2.3 \times 10^{-5}$ cm² sec⁻¹ (from refs. 12 and 13), and $D_s = 2D_m/3$. The major contribution to these values of H_t arises from the eddy-diffusion term with $\lambda = 1.0$, and of course there is no need to consider the polydispersity term for toluene. The experimental values of H for toluene in Fig. 2 are 21.5 μ m and 27.5 μ m at $u = 1.7$ and 5.0 mm sec⁻¹, respectively. Since the eddy-diffusion term is the only dispersion term contributing to chromatogram broadening of a non-permeating polystyrene standard for this range of u , the representation of the mobile-phase dispersion of a high polymer by H_t is not unreasonable in the estimation of $[\overline{M}_w/\overline{M}_n]_T$ with use of eqn. 2.

CONCLUSIONS

The general expression proposed for the plate height of small molecules may be simplified for high polymers, so that the only contribution to plate height arising from mobile-phase dispersion is from the eddy-diffusion mechanism. Consequently, the contribution to plate height from solute mass transfer in the stationary phase may be determined from the flow-rate dependence of experimental data for plate height. Calculation of the diffusion coefficient of polystyrene in the stationary phase from the mass-transfer dispersion term suggests that macromolecular diffusion in the stationary phase in GPC is restricted; this is in agreement with data reported by other workers. The contribution to experimental plate height arising from the polydispersity of polystyrene has been estimated by assuming that the mobile-phase dispersion term for a high polymer may be represented by plate-height data for toluene. Values of the polydispersity of polystyrene standards were in reasonable agreement with theoretical expectation.

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