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## HIGH-PERFORMANCE GEL PERMEATION CHROMATOGRAPHY OF POLYSTYRENE WITH SILICA MICROSPHERES

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### SUMMARY

Gel permeation chromatography separations have been performed with short low capacity columns containing silica microspheres (particle diameter  $\approx 20 \mu\text{m}$ ) having narrow particle size distributions and mean pore diameters in the range 8–120 nm. Plate height data for non-permeating polystyrene standards permitted an evaluation of chromatogram broadening due to mobile phase dispersion. These results together with plate height data for permeating polystyrene standards, tetraphenylethylene and toluene gave an assessment of chromatogram broadening due to mass transfer dispersion as a function of eluent flow-rate and solute molecular weight. It was found that mass transfer dispersion increased at higher flow-rates, the flow-rate dependence increasing as the diffusion coefficient of permeating polystyrene decreased. At very low flow-rates ( $0.05 \text{ cm}^3 \text{ min}^{-1}$ ), mobile phase dispersion is the major contributor to the chromatogram broadening of permeating polystyrene. From theoretical considerations of chromatogram broadening, a relation is derived permitting the approximate determination of the true polydispersity of a permeating polymer at very low eluent flow-rates from the plate height for that polymer and from the plate height arising from mobile phase dispersion. The results show that fast separations may be accomplished in several minutes and that the most precise determinations of polydispersity are obtained at slow eluent flow-rates with separation times of about 1 h.

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### INTRODUCTION

Theoretical treatments of chromatogram broadening predict that column performance depends on flow mechanisms in the mobile phase and on solute mass transfer between the stationary and mobile phases<sup>1</sup>. Experimental studies of the dependence of solute dispersion mechanisms on the solute diffusion coefficient  $D_m$ , on eluent flow-rate  $u$  and on the particle diameter of the column packing  $d_p$  have been performed in separations by gel permeation chromatography (GPC)<sup>2-5</sup>. Čoupek and Heitz<sup>2,3</sup> found good agreement between the theoretical treatments and experimental results for permeating and non-permeating oligomers separating on soft homo-

generously cross-linked organic gels with  $d_p \geq 24 \mu\text{m}$ . Billmeyer and Kelley<sup>4,5</sup> concluded that mobile phase dispersion was the major contributor to chromatogram broadening for cross-linked polystyrene gels with  $d_p \approx 50 \mu\text{m}$  and that mass transfer dispersion was the predominant broadening mechanism for inorganic particles with  $d_p > 100 \mu\text{m}$ . This mass transfer contribution was evaluated by comparing the experimental behaviour of low polymers with non-porous and porous inorganic packings. An alternative procedure is to compare permeating and non-permeating solutes with the same column, and results for low polymers with porous glass ( $d_p \approx 44\text{--}74 \mu\text{m}$ ) have been presented by Giddings *et al.*<sup>6</sup>

Column performance is markedly improved by reducing  $d_p$ . Theory predicts that mobile phase dispersion due to the eddy diffusion mechanism depends on  $d_p$  and that solute dispersion due to mass transfer decreases as the depth of penetration into the stationary phase falls, and therefore as  $d_p$  is reduced<sup>1</sup>. Microparticulate silicas ( $d_p \approx 5\text{--}20 \mu\text{m}$ ) which produce high column efficiencies for small molecules are widely used in high-performance liquid chromatography<sup>7</sup>. Several microparticulate silicas with wide pores have been prepared for high-performance GPC (HPGPC) separations of polymers<sup>8-12</sup>. Silica microspheres for chromatographic separations have been developed at AERE Harwell, and "Spherisorb" (trademark of the United Kingdom Atomic Energy Authority) has given outstanding performance in liquid chromatography<sup>13,14</sup>. Columns of Spherisorb contain rigid spheres with a narrow particle size distribution, so that a bed of particles has good flow properties and a low resistance to fluid flow. Previously, we showed that Spherisorb (mean pore diameter 8 nm) will separate low polymers, and that column efficiency is influenced by particle size and particle size distribution<sup>15</sup>. Although our earlier work demonstrated the dependence of column efficiency on  $d_p$ , our chromatographic conditions were far from optimum. We have now studied column efficiencies for Spherisorb and wide pore silicas ( $d_p \approx 20 \mu\text{m}$ ) having mean pore diameter in the range 30-120 nm with a much improved injection procedure and a constant-flow pump. Initial resolution results for three of the packings have been reported elsewhere<sup>16</sup>. In this paper column efficiency results are described for permeating and non-permeating high polymers as a function of  $u$ , in order to assess the contributions to chromatogram broadening in HPGPC arising from solute dispersion in the mobile phase and during mass transfer. These results show how the mass transfer contribution increases for high polymers as the diffusion coefficient for the solute in the stationary phase falls. These data are extremely useful in determining the optimum experimental conditions for high-speed and high-resolution GPC separations of polymers.

## EXPERIMENTAL

Spherisorb silica S.20.W was supplied by Phase Separations (Queensferry, Great Britain) and three laboratory-prepared macroporous silicas designated H2, H4 and H6 were kindly provided by Dr. J. D. F. Ramsay of AERE Harwell. The silica particles were examined by scanning electron microscopy, and the particle size distribution was determined by Coulter Counter by the method described previously<sup>17</sup>. The silica microspheres were slurry-packed into individual 316 grade seamless stainless-steel columns (20 cm  $\times$  0.3 cm I.D.) with a balanced density of tetrabromoethane and tetrachloroethylene, as in the technique described by Majors<sup>18</sup>. The

packing pressures were 1800, 500 1000 and 750 p.s.i. (1 p.s.i.  $\equiv$  6894.8 N/m<sup>2</sup>) for S.20.W, H2, H4 and H6 columns respectively.

HPGPC separations were performed with a Perkin-Elmer Model 1220 positive displacement syringe pump (flow settings 0.05–6.00 cm<sup>3</sup> min<sup>-1</sup>,  $\leq$  3000 p.s.i., 500 cm<sup>3</sup> capacity). In order to ensure reproducible constant flow of the eluent, the pump was initially run at a high flow-rate until the operating pressure was achieved, and the flow-rate was then reduced to that required<sup>19</sup>. With the pumping system at equilibrium, the retention volume of a solute  $V_R$  was calculated from the travel of the recorder chart paper. An Applied Research Labs. ultraviolet detector (254 nm, cell volume 8  $\mu$ l) was used to detect the solute in the eluent. A steady baseline free of noise and drift was obtained with the detector on maximum sensitivity. The connecting tubing between column and detector was shortened in order to minimise dead volume. A detailed description of the instrumentation requirements for HPGPC separations of polymers is given elsewhere<sup>19,20</sup>. Syringe injection (2  $\mu$ l) through a septum into the top of a column was used for toluene (AnalaR), tetraphenylethylene (TPE) (Aldrich, Milwaukee, Wisc., U.S.A.), and polystyrene standards (Waters Assoc., Milford, Mass., U.S.A. and Pressure Chemical, Pittsburgh, Pa., U.S.A.). The GPC eluent was tetrahydrofuran (BDH, Poole, Great Britain) which was destabilised, stored over calcium hydride for 4 h, distilled from calcium hydride, and degassed by stirring under vacuum before use. Each injected solution had a solute concentration of 0.2% (w/v), except for TPE which was 0.01% (w/v). The injection procedure involved pushing the syringe needle through a porous Teflon disc so that the needle touched the centre of a stainless-steel mesh at the top of the silica packings, as described by Webber and McKerrell<sup>21</sup>.

Plate height,  $H$ , for each solute was calculated from

$$H = L/N \quad (1)$$

where  $L$  is the column length and  $N$  is the plate number which was determined from an experimental chromatogram with the relation

$$N = 5.54 (V_R/w_{0.5})^2 \quad (2)$$

where  $w_{0.5}$  is the width of the chromatogram at half its height. Eqn. 2 assumes a symmetrical chromatogram corresponding to a normal error (or Gaussian) function. All results for  $H$  were the average of at least three injections.

## THEORY

In order to interpret the experimental results for  $H$ , the dependence of  $H$  on the solute dispersion mechanisms contributing to chromatogram broadening is required. In addition, the value of  $H$  determined from an experimental chromatogram for a permeating polymer will contain a contribution from polydispersity, and an approach for relating the experimental and true values of  $H$  has been discussed<sup>22</sup>. Here, the expression for  $H$  considered in an earlier paper<sup>19</sup> will be extended to show how the true polydispersity may be derived from an experimental chromatogram. The experimental  $H$  value is given by

$$H = A + (B/u) + Cu + (\sigma_M^2/L) \quad (3)$$

in which  $A$ ,  $B$  and  $C$  are coefficients depending on several parameters (see later), where term I ( $A$ ) is the eddy diffusion term for solute dispersion in the mobile phase, term II ( $B/u$ ) results from dispersion owing to molecular diffusion in the longitudinal direction in the mobile phase, term III ( $Cu$ ) results from solute dispersion owing to mass transfer, and the fourth term contains the standard deviation  $\sigma_M$  for the true molecular weight distribution. For this last term by analogy with definitions for  $H$ <sup>20</sup>, we may define  $\sigma_M$  (in units of length) in terms of  $\sigma_V$  (in units of retention volume) with

$$(\sigma_M^2/L) = (L \sigma_V^2/V_R^2) \quad (4)$$

where  $\sigma_V$  represents a contribution to the experimental chromatogram.

If it is assumed that the true molecular weight distribution of the polystyrene standards may be represented by a logarithmic normal distribution, then for a permeating polymer the true polydispersity defined as the ratio of the weight average and number average molecular weights  $[\bar{M}_w/\bar{M}_n]_T$  may be calculated from

$$\ln[\bar{M}_w/\bar{M}_n]_T = \sigma_D^2 \quad (5)$$

where  $\sigma_D$  is the standard deviation in terms of  $\ln$  molecular weight<sup>23</sup>. Because the experimental chromatograms for polystyrene standards are almost symmetrical and because the GPC separation gives an almost linear calibration plot of  $\log$  molecular weight *versus*  $V_R$  over the permeation range, the polydispersity may be calculated from  $\sigma_V$  with the relation

$$\ln[\bar{M}_w/\bar{M}_n]_T = \sigma_V^2 D_2^2 \quad (6)$$

where  $D_2$  is the slope of the GPC calibration relation between  $\ln$  molecular weight and  $V_R$ . With eqns. 4 and 6 we can show that eqn. 3 gives

$$H = A + (B/u) + Cu + (L \ln[\bar{M}_w/\bar{M}_n]_T/D_2^2 V_R^2) \quad (7)$$

Since results for permeating and non-permeating solutes as a function of polymer size will be considered, the coefficients  $A$ ,  $B$  and  $C$  may be written out in full<sup>1</sup>, giving

$$H = 2\lambda d_p + (2\gamma D_m/u) + [q R(1 - R)d_p^2 u/D_m] + (L \ln[\bar{M}_w/\bar{M}_n]_T/D_2^2 V_R^2) \quad (8)$$

where  $\lambda$  (close to unity) is a constant characteristic of the packing,  $\gamma$  is a tortuosity factor,  $q$  is a configuration factor, which depends on the shape of the pores in the stationary phase, and  $R$  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 which may be found with a non-permeating polymer.

## RESULTS AND DISCUSSION

The micrographs in Fig. 1 illustrate the regularity of the silica microspheres (particle diameter  $\approx 20 \mu\text{m}$ ). From the particle size distribution, mean particle diameters were calculated with the equations given elsewhere<sup>17</sup> and are shown in Table I. These results confirm the narrow microsphere size distribution for the commercial S.20.W silica, as stated previously<sup>15</sup>. The laboratory-prepared silicas tend to

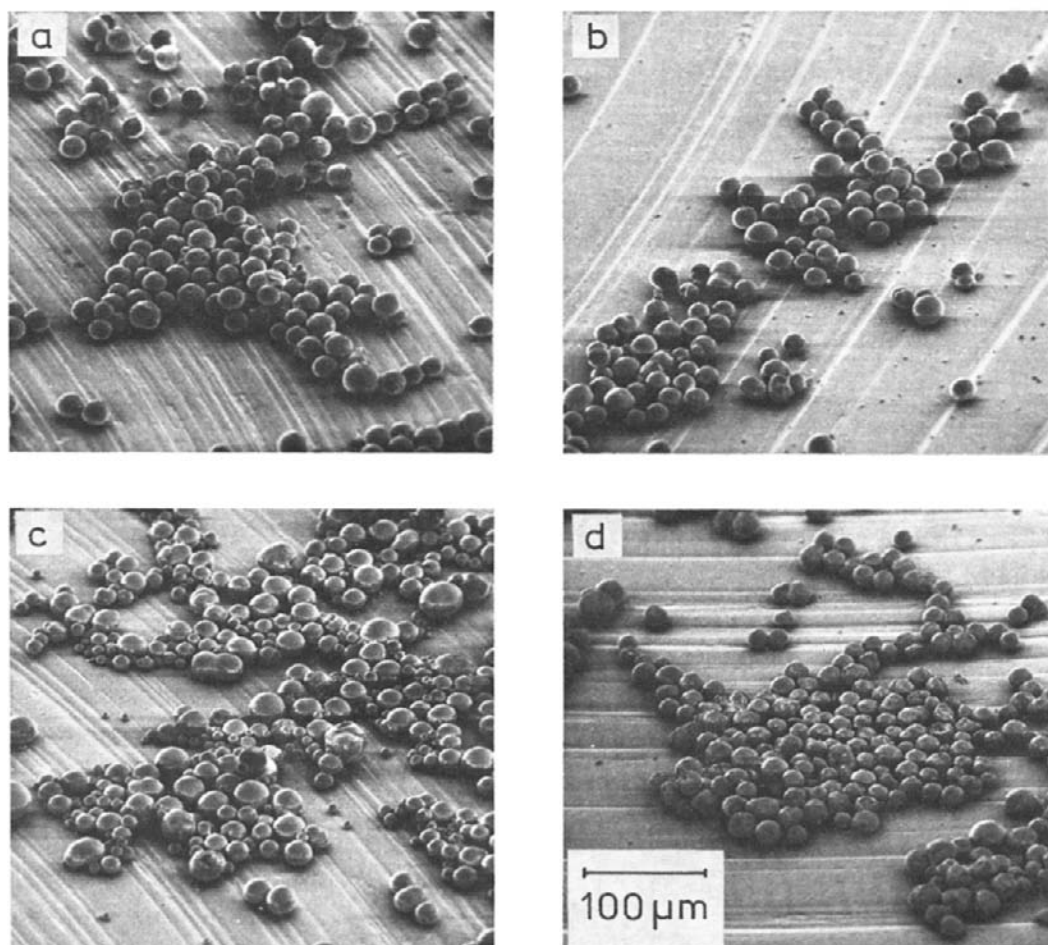


Fig. 1. Scanning electron micrographs of silicas: (a) S.20.W; (b) H4; (c) H6; (d) H2.

TABLE I

NUMBER AVERAGE AND WEIGHT AVERAGE PARTICLE DIAMETERS  $s_n$  AND  $s_w$  AND POLYDISPERSITY OF SILICA PARTICLES

Silica	$s_n$ ( $\mu\text{m}$ )	$s^*$ ( $\mu\text{m}$ )	$s_w$ ( $\mu\text{m}$ )	$s/s_n$	$s_w/s_n$
S.20.W	16.96	17.54	18.78	1.04	1.11
H2	13.92	15.00	16.98	1.08	1.22
H4	12.76	13.74	16.59	1.08	1.30
H6	8.50	10.11	14.17	1.19	1.67

\* Defined as the ratio of the second and first moments (see ref. 17).

be less regular with a wider particle size distribution. The mean particle size and width of the particle size distribution for H2 silica are similar to the characteristics of a fraction (10–15  $\mu\text{m}$ ) of cross-linked polystyrene gel particles<sup>17</sup>. The particle size

distributions of H2 and H4 silicas are very similar but H6 silica has a lower mean particle diameter and a wider particle size distribution.

#### Molecular weight calibration

GPC calibration curves of log molecular weight versus  $V_R$  at an eluent flow-rate of  $0.2 \text{ cm}^3 \text{ min}^{-1}$  are shown in Fig. 2. The shapes of these curves suggest that the silicas have very narrow pore size distributions, a conclusion also reached from nitrogen adsorption isotherms measured at  $77^\circ \text{K}$  (refs. 13, 14). The calibration curves also suggest that the total pore volume of H2, H4 and H6 silicas is about twice the total pore volume of S.20.W, in line with observations on adsorption isotherm data for the macroporous silica microspheres<sup>13,14</sup>. From the calibration curves in Fig. 2, it may be assumed for all the packings that polystyrene standard PS-1987000 is a non-permeating solute and that toluene is a totally permeating solute.

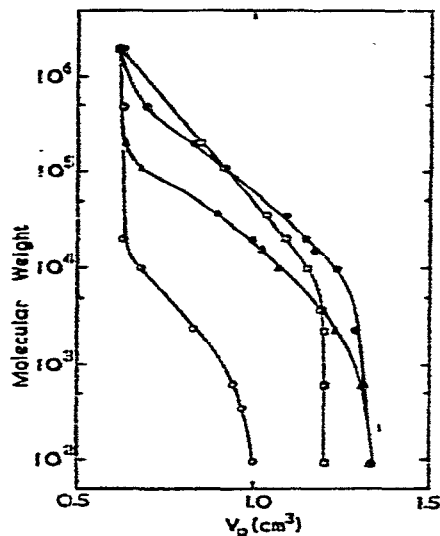


Fig. 2. GPC calibration curves for polystyrene standards, TPE and toluene;  $\circ$ , S.20.W silica;  $\Delta$ , H4 silica;  $\bullet$ , H6 silica;  $\square$ , H2 silica.

#### Silica S.20.W

Curves showing the dependence of  $H$  on eluent flow-rate for S.20.W silica are given in Fig. 3. Values of  $H$  for toluene, TPE and polystyrene standard PS-110000 are about a factor of ten lower than the values reported in an earlier paper<sup>15</sup>. This results from the improved injection procedure, following Webber and McKerrell<sup>21</sup>, in which the solution is injected at the central point of the column, and from the reliable slurry-packing procedure. The solute band will diverge as it progresses down the column, and with the best choice of column diameter  $d_c$  and  $L$ , the solute molecules may never reach the column wall before leaving the column. Since substantial chromatogram broadening may result from solute dispersion at the column wall, the use of "infinite diameter" columns has been advocated in liquid chromatography<sup>24-26</sup>.

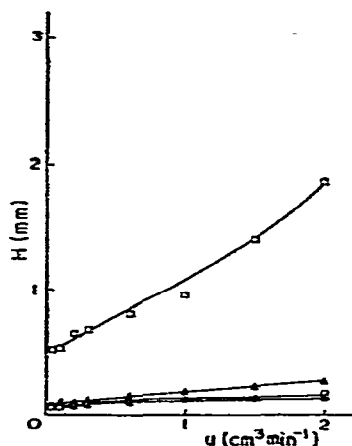


Fig. 3. Dependence of plate height on flow-rate for S.20.W silica: ●, PS-470000 and PS-110000; □, PS-2350; ▲, TPE; ○, toluene.

Several expressions have been proposed for predicting the onset of the "infinite diameter" condition, one criterion being

$$(0.4 d_c/d_p) > (L/d_c) \quad (9)$$

Our columns are therefore close to functioning in the "infinite diameter" mode and the divergence of the solute band down the column will decrease for larger solutes and faster flow-rates. Our choice of column dimensions was mainly determined by the limited quantities of silica samples.

In Fig. 3, it is observed that the two non-permeating polystyrenes having low diffusion coefficients give little variation of  $H$  with flow-rate. For  $u > 0.3 \text{ cm}^3 \text{ min}^{-1}$  the mean value of  $H$  for PS-110000 and PS-470000 is about 0.13 mm and for  $u < 0.3 \text{ cm}^3 \text{ min}^{-1}$  the mean value was about 0.08 mm. Values of  $H$  for PS-470000 were always greater than values for PS-110000. TPE which permeates slightly less pore volume than toluene (Fig. 2), gives a much more significant rise in  $H$  as flow-rate increases than the non-permeating solutes. These observations are in agreement with the general conclusions previously reported<sup>15</sup>. These results may be interpreted in terms of eqns. 3, 7 and 8. For high-molecular-weight polymers with low values of  $D_m$ , term II may be neglected as discussed elsewhere<sup>19</sup>. For non-permeating polymers, term III and the polydispersity term do not arise, and hence  $H$  should be a constant independent of flow-rate as observed in Fig. 3. For toluene, there is no increase in  $H$  as flow-rate decreases, and term II may be neglected. The polydispersity term does not arise for toluene, and so the dependence of  $H$  for toluene on flow-rate suggests that the term for solute mass transfer between the mobile and stationary phases is not very significant. This is explained by the high value of  $D_m$  for toluene in term III in eqn. 8. Kelley and Billmeyer<sup>4,5</sup> have proposed that additional solute dispersion in the mobile phase may arise from a non-uniform flow velocity profile across a column, and they interpreted the much higher  $H$  values for non-permeating polystyrene than for a permeating solute as evidence for a velocity profile effect. In Fig. 3, the  $H$  values

for toluene and the two polystyrenes are very similar, suggesting that the velocity profile effect is reduced considerably. A non-uniform flow velocity effect across a column is expected with particles having a wide size range, because of variable resistance to fluid flow across a column. It is evident in Fig. 1 and Table I that S.20.W silica particles are very regular and have a narrow size distribution. Provided the column is packed carefully, a homogeneous bed of particles should result. The results for toluene and non-permeating polystyrenes with S.20.W silica are similar to  $H$  reported for cross-linked polystyrene gel particles (16–20  $\mu\text{m}$ ) having a spherical shape and a narrow particle size distribution, see Fig. 6 in ref. 17. All these results suggest that optimum performance requires not only a low value of  $d_p$  but a narrow particle size distribution. Small differences between  $H$  values for silica and cross-linked polystyrene gels may arise from a contribution to  $H$  from solute adsorption onto the pore surface, and Giddings *et al.*<sup>6</sup> have identified a small adsorption contribution for solutes in dichloroethane separating on porous glass. This effect has not been considered in eqn. 8.

In Fig. 3, the divergence of the curves for permeating solutes as flow-rate rises suggests a dependence of permeation dispersion on solute diffusion coefficient. The slope of the curve for PS-2350 is about eight times greater than the slope for TPE. This may be interpreted in terms of the mass transfer dispersion term in eqn. 8. Values for  $R(1-R)$  may be calculated from Fig. 2 and are 0.19 and 0.23 for PS-2350 and TPE respectively. The diffusion coefficients, calculated as described elsewhere<sup>19</sup>, are  $3.72 \cdot 10^{-6}$  and  $1.27 \cdot 10^{-5} \text{ cm}^2 \text{ sec}^{-1}$  respectively. Therefore, for permeating solutes the slope of a plot of  $H$  versus  $u$  increases as solute diffusion coefficient decreases, as observed previously for polystyrene standards covering a wide molecular weight range<sup>19</sup>.

The vertical displacement of the curve for PS-2350 with respect to the curves for toluene and TPE results from the polydispersity term in eqn. 8. This term may be evaluated approximately by considering that at very low eluent flow-rates mobile phase dispersion is the major contributor to chromatogram broadening. Since the results suggest that term II is unimportant in our experiments and that the  $H$  curves for toluene and the non-permeating polystyrene standards are very close together in Fig. 3, we propose that the plate height for toluene  $H_t$  could represent the solute dispersion terms in eqn. 8 at the lowest practical flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$ . Consequently, we may assume that  $H$  for a permeating polystyrene standard  $H_{PS}$  at the same flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$  is given approximately by

$$H_{PS} = H_t + (L \ln[\bar{M}_w/\bar{M}_n]_T/D_2^2 V_R^2) \quad (10)$$

in which the second term on the right hand side is for the permeating polystyrene standard. The results in Fig. 3 suggest that term III for toluene at very low flow-rates is extremely small. For a permeating polystyrene term III will be larger because of the lower value of  $D_m$ , but provided mass transfer dispersion is less important than mobile phase dispersion at very low flow-rates, the use of toluene data for  $H_t$  will not be too unreasonable. From the difference between the  $H_{PS}$  and  $H_t$  values at the lowest flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$  in Fig. 3, a value of  $[\bar{M}_w/\bar{M}_n]_T = 1.15$  was evaluated for PS-2350 and is given in Table II. Values of  $D_2$  and  $V_R$  in this procedure were obtained from Fig. 2. The calculated true polydispersity is not unreasonable con-



TABLE II  
POLYDISPERSITIES EVALUATED FROM PLATE HEIGHT DATA  
Eluent flow-rate,  $0.05 \text{ cm}^3 \text{ min}^{-1}$

Column	Polystyrene standard	$H_{PS}$ (mm)	$H_t$ (mm)	$[\bar{M}_w/\bar{M}_n]_T$
S.20.W	PS-2350	0.52	0.062	1.16
H4	PS-9800	0.31	0.070	1.08
H4	PS-35000	0.28	0.070	1.05
H6	PS-35000	0.24	0.110	1.02
H2	PS-35000	0.12	0.110	1.00
H2	PS-200000	0.31	0.110	1.06

sidering the assumptions involved. For a polystyrene prepared by "living" anionic polymerisation, the theoretical value of  $[\bar{M}_w/\bar{M}_n]_T$  for a sample of molecular weight 2350 is 1.04 (ref. 23), and a somewhat higher practical value might be expected because of the rigorous conditions required in the experimental polymerisation technique.

#### Silicas H4, H6 and H2

Curves showing the dependence of  $H$  on eluent flow-rate are shown in Figs. 4-6. It is observed that the non-permeating PS-1987000 and the totally permeating toluene give little or no variation of  $H$  with flow-rate, and therefore the explanations discussed for S.20.W silica also hold for the macroporous silicas. It was reported previously that plate numbers for H4 and H2 silicas were lower than for S.20.W silica<sup>16</sup>, and this is supported by the plots in Figs. 4-6 which lie at higher values of  $H$  for toluene and PS-1987000 than the curves in Fig. 3. Since the value of  $H$  for the non-permeating polystyrene in Figs. 4-6 is higher than in Fig. 3, we may conclude from eqn. 8 that the columns of H4, H6 and H2 silicas have higher mobile phase dispersion than the S.20.W column. This follows because we may neglect, as previ-

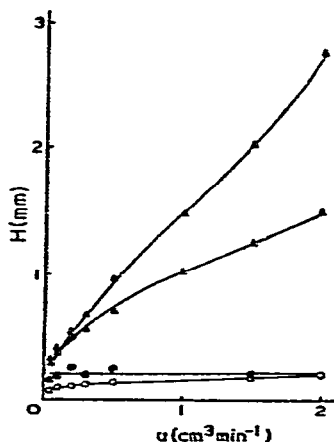


Fig. 4. Dependence of plate height on flow-rate for H4 silica: ●, PS-1987000; ▲, PS-35000; ▲, PS-9800; ○, toluene.

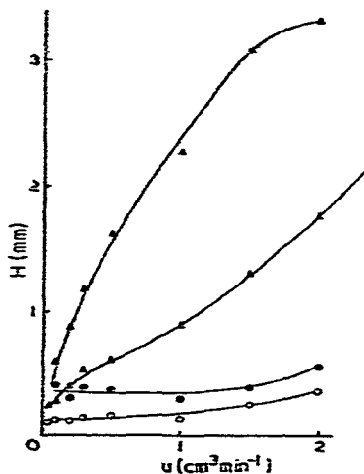


Fig. 5. Dependence of plate height on flow-rate for H6 silica: ●, PS-1987000; ▲, PS-200000; △, PS-35000; ○, toluene.

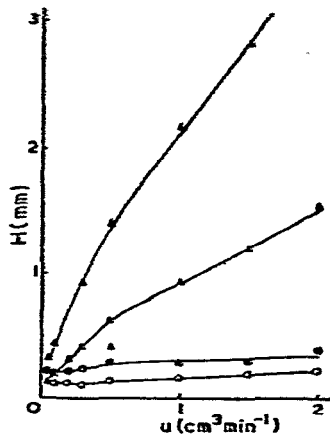


Fig. 6. Dependence of plate height on flow-rate for H2 silica: ●, PS-1987000; ▲, PS-200000; △, PS-35000; ○, toluene.

ously discussed, terms II and III and the polydispersity term when considering eqns. 3, 7 and 8 for a non-permeating polymer. This increase in mobile phase dispersion for H4, H6 and H2 silicas may be explained by the less regular particles (Fig. 1) and the wider particle size distributions (Table I) giving a heterogeneous bed of particles so that multiple non-uniform flow-paths are generated. The values of  $H$  for toluene and PS-1987000 with the H6 silica column are somewhat higher than the values for the H4 and H2 silicas, as expected from the high value of  $s_w/s_n$  in Table I. The increase in  $H$  because of the wide particle size distribution of silica H6 will tend to be balanced by a decrease in  $H$  due to the lower value of  $d_p$ .

In Fig. 2, it appears that the H6 and H2 silicas separate a similar range of molecular sizes, although the silicas may have somewhat different pore size distributions. The dependence of  $H$  on flow-rate for the permeating polystyrenes PS-35000 and PS-200000 is similar for these two silicas in Figs. 5 and 6 suggesting that the mass transfer characteristics of the two columns are the same. The slopes of these curves at  $u \leq 1.5 \text{ cm}^3 \text{ min}^{-1}$  again show that  $H$  increases on permeation and that permeation dispersion increases for macromolecules with lower diffusion coefficients. At the lowest flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$  in Figs. 5 and 6,  $H$  for PS-35000 is lower than  $H$  for PS-1987000 and not much larger than  $H$  for toluene. If the difference between  $H$  for PS-35000 and toluene is assumed to be the polydispersity term in eqns. 3, 7 and 8, then  $[\overline{M}_w/\overline{M}_n]_T$  may be calculated for PS-35000 with eqn. 10. Values calculated from Figs. 5 and 6 are shown in Table II. The same procedure was used to calculate  $[\overline{M}_w/\overline{M}_n]_T$  for PS-9800 and PS-200000 from Figs. 4 and 6 respectively. Fig. 6 was preferred for PS-200000 since the calibration curves in Fig. 2 suggest that H2 silica gives a better resolution of the high-molecular-weight chains and therefore a more reliable chromatogram. The average of the values of  $[\overline{M}_w/\overline{M}_n]_T$  for PS-35000 in Table II may be regarded as acceptable. An even lower value of the true polydispersity

is expected for columns with smaller particles<sup>19</sup>. Although  $H_{PS}$  values for PS-35000 in Table II are similar at the lowest practical flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$  for the three silica columns, the slope of the curve of  $H$  versus  $u$  in Fig. 4 is higher than for H6 and H2 silicas. This cannot be explained by the value of  $R(1-R)$  and may possibly be explained by differences in the internal pore structure between H4 silica and the H6 and H2 silicas. Another possibility is that  $D_m$  for PS-35000 is lower for H4 silica than for H6 and H2 silicas, since PS-35000 elutes closer to the exclusion limit with H4 silica so that there may be steric restriction as the polymer diffuses in a tight pore. We have assumed that  $D_m$  is for a polymer in free solution, but the diffusion coefficient may change depending on pore geometry and size when a polymer enters the stationary phase.

The displacement of  $H$  for PS-1987000 with respect to  $H$  for toluene in Figs. 4-6 might suggest that mobile phase dispersion is polymer size dependent, since the curves in Fig. 3 for toluene and the non-permeating polystyrenes are very close together. However, the higher values for PS-1987000 are likely to result from the practical difficulties associated with ultra-high-molecular-weight polymers which may have additional chromatogram broadening owing to experimental concentration, viscosity and degradation effects. Since the permeating polystyrenes in Figs. 4-6 have molecular weights similar to or below the molecular weights of the non-permeating polystyrenes in Fig. 3, it appears reasonable to use  $H$  data for toluene in eqn. 10 for the permeating polystyrenes in Figs. 4-6.

The values of the true polydispersity in Table II were calculated from  $H$  data with eqn. 10. This equation involves several assumptions and the determination of experimental values of  $H$  involves the standard deviation  $\sigma$  of the chromatogram. A much more reliable evaluation of polydispersity results from the use of all the chromatogram, as in the computer program of Pickett *et al.*<sup>27</sup>. This experimental polydispersity  $[\overline{M}_w/\overline{M}_n]$  may be related to  $[\overline{M}_w/\overline{M}_n]_T$  if it is assumed that the chromatogram and the molecular weight distribution are represented approximately by a logarithmic normal function. The experimental value of  $H$  is given by  $\sigma^2/L$  (ref. 1). It follows from eqns. 4, 5 and 6 that eqn. 8 may be transformed to

$$\ln[\overline{M}_w/\overline{M}_n] = \frac{D_z^2 V_R^2}{L} \{2\lambda d_p + (2\gamma D_m/u) + [qR(1-R)d_p^2 u/D_m]\} + \ln[\overline{M}_w/\overline{M}_n]_T \quad (11)$$

It follows from eqn. 10 that the chromatogram broadening terms in eqn. 11 may be evaluated from experimental  $H$  data for toluene at low  $u$ . Consequently,  $[\overline{M}_w/\overline{M}_n]_T$  may be calculated from  $[\overline{M}_w/\overline{M}_n]$  determined experimentally from the chromatogram of a permeating polymer at low  $u$ . The drawback of this procedure is that even at low flow-rates the mass-transfer term for a permeating polymer will be somewhat higher than for toluene. Therefore, estimates of  $[\overline{M}_w/\overline{M}_n]_T$  are likely to be too high.

## CONCLUSIONS

The similarity of  $H$  values for non-permeating polystyrenes and toluene suggests that mobile phase dispersion is the major cause of chromatogram broadening for small molecules. Because mass transfer dispersion is quite low for small molecules, high-resolution GPC separations of oligomers and low polymers may be performed

at fast flow-rates. High-performance will not, however, be obtained from micro-particulate packings if the particles are not regular and do not have a narrow size distribution. Mass transfer dispersion becomes much more important for high polymers and increases as polymer diffusion coefficient decreases and therefore as molecular size increases. Consequently, extensive chromatogram broadening will occur for permeating high polymers at fast flow-rates. Efficient separations of high polymers, giving  $H$  similar to those of small molecules, are only obtained at extremely low flow-rates, for example below  $0.1 \text{ cm}^3 \text{ min}^{-1}$ . Mass transfer dispersion is then much reduced, and it is possible to calculate approximately the polydispersity of a permeating polystyrene standard from its  $H$  value and the  $H$  for toluene which is close to the  $H$  value arising from mobile phase dispersion determined with a non-permeating polystyrene standard. For the single columns investigated, separations at a low flow-rate of  $0.05 \text{ cm}^3 \text{ min}^{-1}$  may be completed in under 30 min. For routine work four columns in series with samples of S.20.W, H4, H6 and H2 silicas in each column, or a single column with length 80 cm containing all four silicas, would be necessary, giving efficient separations for permeating high polymers in under 2 h. This separation time is not unreasonable, and accurate determinations of molecular weight distribution and polydispersity will then be obtained at the extremely low flow-rate. The main practical disadvantage is the determination of retention volume for short narrow columns, but the precision may be optimised with an accurately controlled constant-flow pump. Clearly, separations for a single column may be performed in about 1 min and for a series arrangement of four columns in under 5 min, but the chromatogram broadening for high polymers is extensive, giving inaccurate values for polydispersity.

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