High performance gel permeation chromatography of polystyrene

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Microparticulate crosslinked polystyrene packings in short columns have been investigated with high performance liquid chromatography instrumentation. Reliable molecular weight data for six polystyrene standards having narrow molecular weight distributions and for a polystyrene having a broad distribution have been obtained by optimizing the injection procedure, using a constant flow pump, and incorporating an internal standard into each injected solution. Experimental determinations of the dependence of the polydispersity for polystyrene standards on eluent flow rate and polymer diffusion coefficient were in agreement with a relation predicted from theoretical considerations of chromatogram broadening. Because of the dependence of chromatogram broadening on polystyrene molecular weight, high efficiency separations for high polymers were only obtained at low eluent flow rates. For low polymers, high efficiency separations may be performed at fast eluent flow rates. It was concluded that accurate molecular weight distributions can only be determined from chromatograms obtained at low eluent flow rates, which was supported by experimental measurements of polydispersity on polystyrene sample prepared by a radical polymerization at low monomer conversion. A differential weight distribution calculated from an experimental chromatogram for the polydisperse polystyrene determined at the lowest eluent flow rate (0.1 cm^{3}min^{-1}) was compared with distributions predicted theoretically for polystyrenes prepared by radical polymerization. It was concluded that the experimental distribution contained a small contribution from chromatogram broadening and that most of the radicals in the polymerization of styrene terminated by combination.

INTRODUCTION

The crosslinked polystyrene gels first described by Moore[1] are widely used for polymer fractionation by gel permeation chromatography (g.p.c.). After the development of a commercial g.p.c. instrument[2], it was shown that reliable and reproducible chromatograms could be obtained, so that the g.p.c. technique is now employed for routine characterization and for quality control measurements. The g.p.c. technique developed over a decade ago consisted of a series arrangement of columns (7.5 mm internal diameter). The preferred total column length was 488 cm, giving a column efficiency of $\sim$70 plates min^{-1} and a separation time of $\sim$3 h at an eluent flow rate of 1 cm^3 min^{-1}. Subsequent experiments[3-5] suggested that such a column efficiency results in gel particles having a mean particle diameter $d_p \sim$ 40 μm.

It follows from theories for chromatogram broadening that column efficiency is raised by reducing d_p[6]. Although column efficiency decreases at higher flow rates, this change is less important for practical flow rates than the increase in column efficiency obtained for particles with $d_p < 20 \mu$m. Columns of such microparticulate crosslinked polystyrene packings have been produced for high speed g.p.c.[7-11]. Typically, flow rates in the range 1–4 cm^3 min^{-1} are employed with a total column length of 120 cm (7.5 mm internal diameter). Therefore, the separation time may be reduced by factors of up to 16 times, giving separations in < 0.5 h with a column efficiency $\sim$700 plates min^{-1}.

Whilst there has been a considerable improvement in speed, this may not be accompanied by an increase in resolution because of the reduction in column length[12]. Furthermore, accurate measurement of retention volume. V, is an essential part of molecular weight calculations from chromatograms. and errors in measuring low volumes from short columns can lead to incorrect values of average molecular weights[13]. Consequently, there has been a tendency towards monitoring retention times, but this requires a precise constant flow pump if significant errors are to be avoided[14]. It has been suggested that errors can further be minimized by incorporating an internal standard into each injected solution[15]. However, high performance separations will not be obtained if the experimental conditions are badly chosen, and Mori[16] has demonstrated that operational variables such as flow rate, injection volume and sample concentration must be optimized.

Our work on high performance gel permeation chromatography (*HP*-g.p.c.) was initiated because of a need for precise determinations of molecular weight distribution, number-average and weight-average molecular weights, $\bar{M}_n$ and $\bar{M}_w$ respectively, and polydispersity $\bar{M}_w/\bar{M}_n$. A pessimistic view on the work described in the previous two paragraphs might suggest that the faster separations could produce less accurate molecular weight distributions than the slower separations with longer columns. A major drawback of separations produced with columns of larger particles ($d_p \sim$ 40 μm) is the magnitude of chromatogram broadening, so that broadening corrections must be applied in order to calculate the

true molecular weight distribution[17-19]. Unfortunately, broadening corrections do not always produce reliable distributions and have often been omitted from many publications in which distributions and average molecular weights have been calculated from gel permeation chromatograms. If the experiments with microparticulate packings in shorter columns were free of practical difficulties and were performed at low flow rates, then very efficient separations are predicted to occur. Such very high efficiency columns could produce minimum chromatogram broadening so that errors arising from omitting the broadening correction are not significant for polydisperse high polymers, giving accurate values for $\bar{M}_n$ and $\bar{M}_w$.

In this paper we describe in detail our instrumentation and procedures for *HP*-g.p.c. in order to achieve the optimum reproducibility and accuracy. Narrow distribution polystyrene standards have been studied as a function of eluent flow rate and molecular weight. The dependence of column efficiency on the diffusion coefficient D_m of polymers has not been widely studied for microparticulate packings ($d_p \sim 10\,\mu m$), and our results suggest that substantial chromatogram broadening will occur for high polymers at fast flow rates. A polydisperse polystyrene prepared under carefully controlled polymerization conditions has also been examined as a function of eluent flow rate, and the molecular weight distribution determined by *HP*-g.p.c. at the lowest flow rate, i.e. maximum column efficiency, has been compared with a distribution predicted from the polymerization mechanism. Surprisingly, this method of improving the accuracy of determinations of molecular weight distribution has received little attention.

EXPERIMENTAL

Materials

Toluene (Analar), tetraphenylethylene (Aldrich), and polystyrene standards (Waters Associates and Pressure Chemical) were used as received. The g.p.c. eluent was tetrahydrofuran (BDH Chemicals) which was destabilized, stored over calcium hydride for 4 h, distilled from calcium hydride, and degassed by stirring under vacuum before use.

A polydisperse polystyrene PSGY2 was prepared by a vacuum line procedure. Styrene (Fisons), dried over calcium hydride for two weeks, was degassed by a series of freeze–thaw cycles under vacuum. It was then vacuum distilled into a calibrated tube already containing α,α'-azobisisobutyronitrile (AZBN) (BDH Chemicals) which had been recrystallized twice from methanol. The styrene (18.4 g) was degassed again, and the tube was sealed after freezing the styrene. The AZBN (0.09g) was dissolved on thawing and the tube was then placed into a thermostatically controlled water bath at 333K. After 1 h the tube was withdrawn from the bath, broken open, and the contents poured into 1 dm^3 of cold methanol. The precipitated polymer was washed 5 times with 200 cm^3 methanol. The polystyrene was dried to constant weight at room temperature at < 15 mm pressure and was then isolated by freeze-drying from benzene. The yield was 1.03 g of polystyrene, corresponding to a conversion of 5.1%.

Osmometry

Osmotic pressure measurements were performed with a Hewlett–Packard Model 502 high speed membrane osmometer at 290.5K with toluene as solvent. Four concentrations of polystyrene PSGY2 in toluene were employed over the range 2–10 g dm^{-3}. The first power of the reduced osmotic pressure was extrapolated linearly to zero concentration and the number-average molecular weight was calculated from the intercept. The value of $\bar{M}_n$ for polystyrene PSGY2 was 76 000.

Gel permeation chromatography

A constant, reproducible, pulse-free eluent flow rate was obtained with a Perkin–Elmer Model 1220 positive displacement syringe pump, flow settings 0.05–6.00 cm^3 min^{-1}, $\leqslant$ 3000 lbf/in^2 (1 lbf/$in^2 \equiv 6894.8$ N/m^2), 500 cm^3 capacity. Reproducible flow is important in *HP*-g.p.c. as the much smaller retention volumes are not easily measured accurately by syphon or drop counter. Many workers have assumed constant flow rate and measured retention volume as a time base along a recorder chart paper; however, this approach must be used cautiously because even small fluctuations in flow rate may result in large errors when determining average molecular weights of polymers. For example, flow rate repeatability must be better than 0.3% otherwise errors in $\bar{M}_n$ and $\bar{M}_w$ will be ~6%[14]. Syringe pumps do not give immediate steady flow conditions owing to the compressibility of the mobile phase[20,21]. In this work, 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. When this pumping system had reached equilibrium the flow rate error was below 0.3%. When the reservoir required refilling, the columns were isolated from the pump by a valve. Any air present in the system after refilling was purged out of an outlet in the pumping system before opening the columns to the solvent again. The minimum volume of solvent remaining in the pump after refilling and establishing constant flow conditions was 300 cm^3 for the highest flow rate. This volume is adequate for *HP*-g.p.c. systems having small columns which generally have a total permeating volume of <50 cm^3. The downtime involved in refilling and establishing constant flow conditions was not critical.

In all experiments at room temperature, the retention volume was calculated from the chart paper travel because of the accuracy of the pump flow rate. The effect of variations in flow rate can be magnified by fluctuations in chart speed. The response time of the detector to give a signal to the chart recorder is also important, as is the time for this signal to be recorded by the chart recorder. All of these errors are small in comparison with the errors which may arise from operating the pump under non-equilibrium conditions. In order to increase the reliability of V values, as internal standard, tetraphenylethylene (TPE), was added to each solution. This procedure was studied by Patel and Stejny[22,23] and has been investigated for *HP*-g.p.c. by Kohn and Ashcraft[15]. The retention volume of TPE was assigned to 100% and the polystyrene standards were measured as a ratio to this. TPE was not completely satisfactory as an internal standard for the standard PS-600 because the tails of the two peaks overlapped. To check the assignment of V for PS-600 in terms of 100% TPE, independent chromatograms for TPE and PS-600 were obtained from two separate injections. The chromatogram of the mixture was resolved into two components according to the independent peaks, permitting the assignment of V for TPE (100%) and PS-600 (in terms of TPE) in the mixture. This method of determining V with reference to 100% TPE was used in the determination of peak heights for the calculation of average molecular weights.
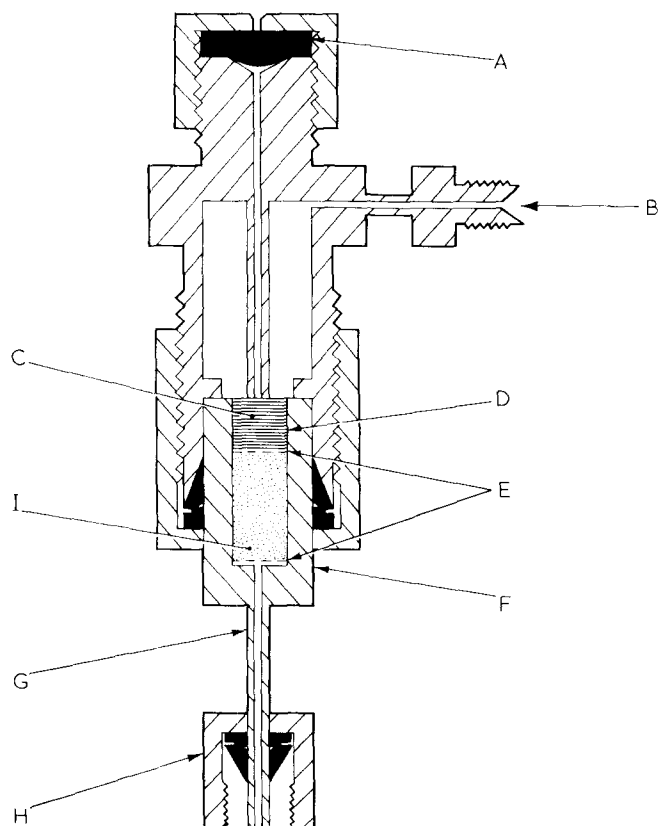
Figure 1 G.p.c. injector. A, PTFE backed silicone rubber septum; B, mobile phase from pump; C, PTFE plug (40 μm pore size); C, 3 x 12 mm pocker; E, woven s/s mesh (5 μm pore size); F, 18 x 6.35 mm o.d. s/s tubing; G, 15 x 1.6 mm o.d. s/s tubing; H, nut for 1.6 mm o.d. tubing; I. Spherisorb S.20

Four μStyragel columns each containing a gel of different pore size (in the order of 10^5, 10^4, 10^3 and 10^2 nm, Waters designation) were obtained from Waters Associates Inc. Hartford, Northwich, Cheshire, England. The gel particles have a diameter of about 10 μm. Each column (30 x 0.76 cm internal diameter) was connected in series in the order of decreasing pore size with low dead-volume tubing (5 x 0.025 cm internal diameter). No corrections were made for instrumental dead volume, since it was considered that this volume was too small to measure accurately.

An Applied Research Laboratories ultra-violet detector (254 nm cell volume, 8 μdm³) was used to detect solute in the eluent. The tubing leading to the detector cell was shortened so that the last column was connected to the detector with the minimum of dead volume. Maximum detector sensitivity for polystyrene is obtained with an ultra-violet detector. Consequently, a smaller quantity of solute may be injected, thus minimizing chromatogram broadening owing to viscous fingering and column overload which were assumed to be unimportant. A steady baseline free of noise and drift was obtained with the detector on maximum sensitivity at all times.

The injection head was attached above the end-fitting of the first column, so that the sample could be injected into the solvent flowing into the top of the column. Solutions were injected through a septum arrangement similar to that described previously[5]. In *Figure 1* the pocket D was modified to 12 mm in length x 3 mm in diameter. A woven stainless steel mesh (5 μm pore size) was placed at the bottom of the pocket and supported particles of Spherisorb S.20W. (Phase Separations) which were covered by a second stainless steel

mesh. The porous Teflon disc (40 μm pore size) rested in the top of the pocket, as shown previously[5]. Injections were performed with a 100 μdm³ Hamilton high pressure syringe No. 1710 (pressure limit ≤1500 lbf/in²) by pushing the syringe needle through the porous Teflon disc so that the needle touched the centre of the upper stainless steel mesh. The porous Teflon disc and the Spherisorb particles acted as filters for septum fragments which may block the connecting tubing (0.025 cm internal diameter) between the injector and the column inlet. All the PTFE-backed silicone septa were replaced after 15–20 injections. The injection head could be used for injection with column pressures up to 2000 lbf/in². Both the injected volume and the solute concentration were lower than the values recommended by Mori[16] for *HP*-g.p.c. separations. In the calibration of the individual columns, the injected volume was 10 μdm³, whereas an injected volume of 40 μdm³ was used for the series arrangement of four μStyragel columns apart from polystyrene PSGY2 for which the injected volume was 80 μdm³. For low molecular weight solutes up to PS-35 000, the solute concentration was 0.2% (w/v). For polystyrenes PS-110 000, PS-200 000, PS-470 000, PS-1 987 000, and PSGY2, the solute concentrations were 0.15, 0.13, 0.1, 0.1 and 0.2% (w/v), respectively. All the solutions of polystyrene standards contained TPE (0.01% w/v) as internal standard. The concentration of TPE was 0.005% w/v for solutions of polystyrene PSGY2. In all injections the injection time was negligible (<0.5 s) compared with the time taken for the sample to elute from the column or columns.

Calculations of molecular weight distribution, average molecular weights and polydispersity from the chromatograms of the polystyrene standards and polystyrene PSGY2 were performed by the procedure of Pickett. Cantow and Johnson[24]. The computer program based on that of Pickett and coworkers is described elsewhere[25]. All results presented are the average of three or more determinations under identical conditions.

INTERPRETATION OF CHROMATOGRAMS

A measure of the efficiency of a chromatography column is the height equivalent to a theoretical plate or plate height H[6]. The plate height for an experimental chromatogram is calculated from the expression:

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

where L is the column length and N is the plate number which may be determined from:

$$N = 5.54 \left(\frac{V}{w_{0.5}} \right) \qquad (2)$$

where $w_{0.5}$ is the width of the chromatogram at half its height. Equation (2) was preferred to the tangent method[26] because of greater precision. Equation (2) assumed a symmetrical chromatogram corresponding to a normal error (or Gaussian) function. For a polydisperse polymer, a symmetrical chromatogram, obtained when the calibration relation between log (molecular weight) and V is linear, is represented by the logarithmic normal function. This is a reasonable observation for polymers with a narrow molecular weight distribution[27].

One of the most widely used relations for predicting column efficiencies, both in gas chromatography and in
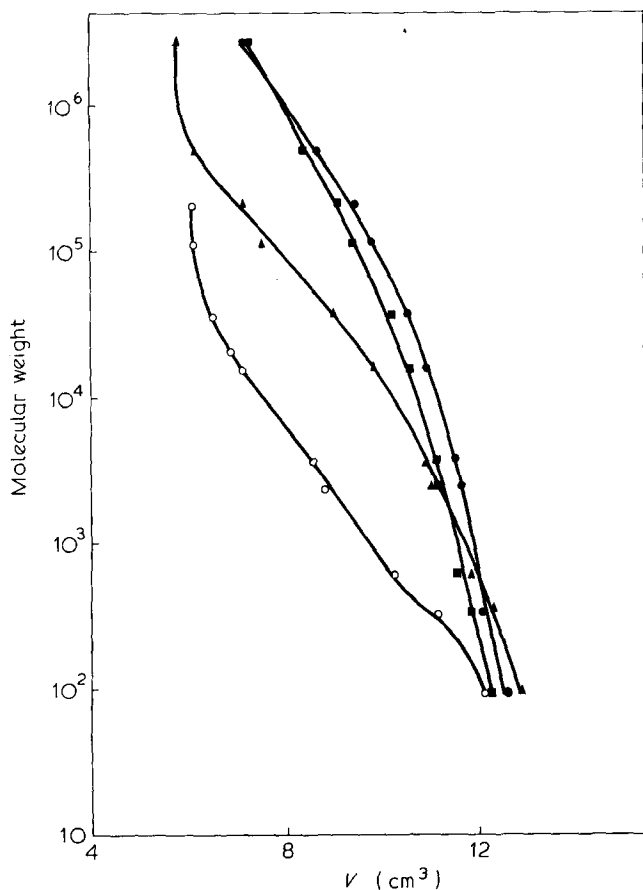
Figure 2 G.p.c. calibration curves for polystyrene standards with crosslinked polystyrene gel columns: ■, 10^5 nm; ●, 10^4 nm; ▲, 10^3 nm; ○, 10^2 nm

liquid chromatography, is the equation developed by van Deemter and coworkers[28] from a mass balance approach:

$$H = A + (B/u) + Cu \tag{3}$$

where u is the eluent flow rate, A in term I is a constant for solute dispersion arising from eddy diffusion in the mobile phase, B in term II is a constant for dispersion from molecular diffusion in the longitudinal direction in the mobile phase, and C in term III is a constant for solute dispersion due to resistance to mass transfer. The plate height may be thought of as the rate of change of peak (or solute zone) variance (in units of length) relative to the distance migrated L[6]. The variance is the square of the standard deviation σ^2, so that H is defined by:

$$H = \sigma^2/L \tag{4}$$

If there are several solute dispersion mechanisms contributing to chromatogram broadening, as represented by equation (3), and if these mechanisms are independent of each other, it follows from the laws of statistics that the variance of the chromatogram will be the sum of the variances associated with the individual mechanisms, i.e.:

$$H = \Sigma \sigma^2/L \tag{5}$$

For a polydisperse polymer, Hendrickson[29] expressed the width of a chromatogram in terms of several variables, including the molecular weight distribution of the polymer and chromatogram broadening arising in the column (or

columns) from solute dispersion mechanisms. It follows that according to equation (5) the standard deviation σ_0 from an experimental chromatogram may be expressed by:

$$\sigma_0^2 = \sigma_I^2 + \sigma_{II}^2 + \sigma_{III}^2 + \sigma_M^2 \tag{6}$$

where σ_I, σ_{II} and σ_{III} follow from the solute dispersion terms in equation (3) and σ_M in units of length is the standard deviation for the true molecular weight distribution of the polymer. Chromatogram broadening owing to extra-column effects will be neglected. In equation (3), the constants A, B and C contain several parameters, including D_m and d_p[6]. We shall hold d_p fixed but shall study the dependence of H on D_m. Therefore, from equations (3), (5) and (6) it follows that the experimental plate height is given by:

$$H = A + [B_{II}D_m/u] + [C_{III}R(1 - R)u/D_m] + [\sigma_M^2/L] \tag{7}$$

in which R is the retention ratio, defined by V_0/V where V_0 is the interstitial (or void) volume of the column, and A, B_{II} and C_{III} are considered to be constants. A procedure allowing for the polydispersity in the determination of plate height has been described by Knox and McLennan[30]. We shall assume that the distribution of the polydisperse polymer may be represented by a logarithmic normal function[31]. The relation between the true polydispersity $[\bar{M}_w/\bar{M}_n]_T$ and the standard deviation σ_D (in terms of $\ln M$ where M is molecular weight) which may be related to σ_m is:

$$\ln[\bar{M}_w/\bar{M}_n]_T = \sigma_D^2 \tag{8}$$

RESULTS AND DISCUSSION

Polystyrene standards

The calibration curves for each of the columns at an eluent flow rate of 1 cm^3 min^{-1} are shown in *Figure 2*. The 10^4 and 10^5 nm columns have similar pore size distributions. Plate numbers for the totally permeating toluene were determined for the individual columns and were in the range 6000–9500 plates/ft. All the remaining experiments were performed with the four columns in series.

A calibration curve for the set of four μStyragel columns at an eluent flow rate of cm^3 min^{-1} is shown in *Figure 3*. The position of the calibration curve was hardly altered by flow rate over the range 0.1–2.0 cm^3 min^{-1}. Values of H for the polystyrene standards were determined from the chromatograms with equations (1) and (2) as a function of flow rate, and the data are plotted in *Figure 4*. It is clear that apart from the totally permeating solute toluene, and also polystyrene PS-3600, no minimum in the plot of H versus u is observed at flow rates above 0.1 cm^3 min^{-1}. For polystyrenes with molecular weights above 10 000, it is apparent in *Figure 4* that the slope of each curve exhibits a trend as the molecular weight of the polystyrene standard increases. On the other hand the value of H at the lowest or the highest flow rate does not vary in any clear way with the molecular weight, which may be explained after inspecting equations (7) and (8) by the observation that the polydispersities of the six polystyrene standards vary from sample to sample. Values of H for the highest column efficiency ($u = 0.1$ cm^3 min^{-1}) and for a flow rate of 1 cm^3 min^{-1} are given in *Table 1* for the 6 polystyrene standards.

Values of $\ln[\bar{M}_w/\bar{M}_n]$ calculated from the chromatograms of the polystyrene standards for two flow rates are also given in *Table 1*. Values of $\ln[\bar{M}_w/\bar{M}_n]$ for all experimental flow rates are plotted *versus* flow rate in *Figure 5*. It can be observed that the relative positions of the curves for the 6 polystyrene standards are somewhat different from the positions in *Figure 4*, a tendency which is also suggested when comparing data in *Table 1*. This may be explained by the suggestion that the polydispersity is calculated from the whole chromatogram whereas H is deduced from the width of the chromatogram at half-height. The latter calculation will not allow for chromatogram tails which are non-Gaussian. The proportion of non-Gaussian tailing may vary among the polystyrene standards. The dependence of the slope of each curve D_3 in *Figure 5* on molecular weight is apparent for the linear region at flow rates $\leqslant 1.0\ \mathrm{cm}^3\ \mathrm{min}^{-1}$. Therefore, the trend observed in *Figure 4* is confirmed. This suggests that equation (7) may be expressed in terms of polydispersity. We therefore propose empirically from equations (7) and (8) that the polydispersity $[\bar{M}_w/\bar{M}_n]$ calculated from an experimental chromatogram is given by:

$$\ln[\bar{M}_w/\bar{M}_n] = k\{A + [B_{\mathrm{II}}D_m/u] + [C_{\mathrm{III}}R(1-R)u/D_m]\}$$

$$+ \ln[\bar{M}_w/\bar{M}_n]_T \tag{9}$$

From the approach of Knox and McLennan[30] it may be shown that k is directly proportional to V^2, assuming a linear calibration curve of $\log M$ *versus* V and a constant value of L. Since V^2 increases as polymer size decreases, we do not have to consider k as a variable in the interpretation of the molecular weight dependence of the slopes of the curves in *Figure 5*.

The molecular weight dependence of D_3 may be interpreted in terms of equation (9). We need to establish that term I which results from eddy diffusion in the mobile phase may be regarded as a constant independent of flow rate Billmeyer and Kelley[32] reported for crosslinked polystyrene
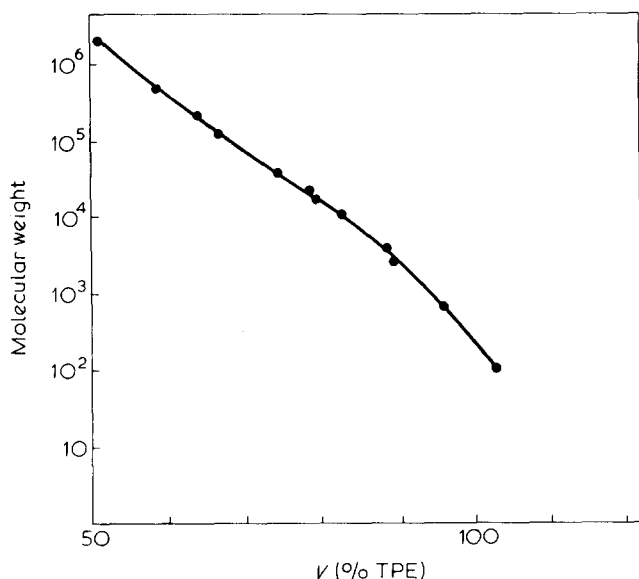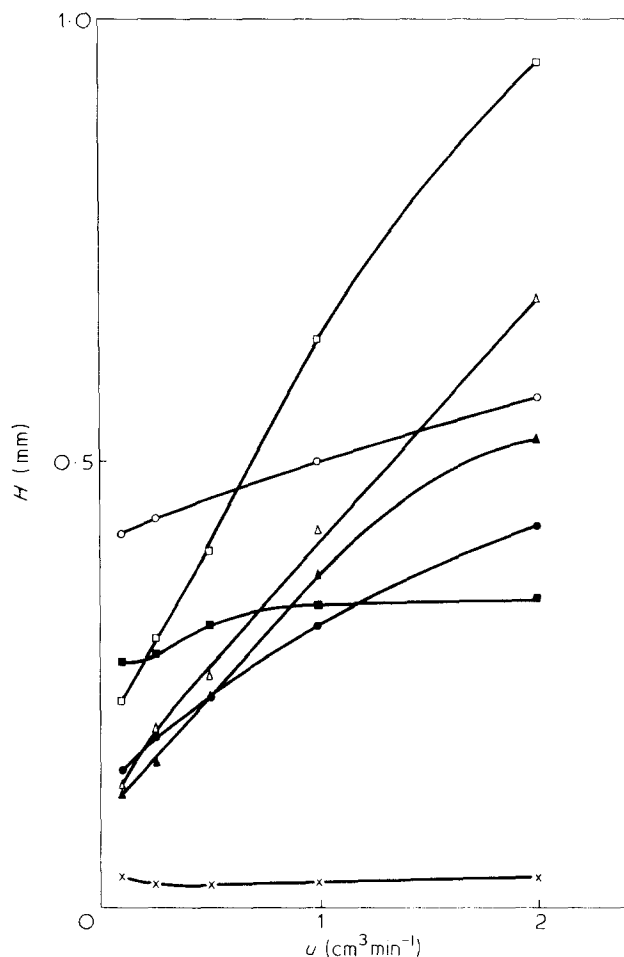

Figure 3 G.p.c. calibration curve for polystyrene standards with four crosslinked polystyrene gel columns in series

Figure 4 Dependence of plate height on eluent flow rate for polystyrene standards. □, PS—470 000; △, PS—200 000; ▲, PS—110 000; ●, PS—35 000; ○, PS—9800; ■, PS—3600; X, toluene

Table 1 Column efficiency, resolution and polydispersity of polystyrene standards

| Polystyrene	u (cm³ min⁻¹)	N (plates ft⁻¹)	H (mm)	R_{sp}	$\bar{M}_w/\bar{M}_n$	$\ln[\bar{M}_w/\bar{M}_n]$
PS—3600	0.1	4500	0.271	2.42	1.115	0.109
	1.0	3620	0.337	2.17	1.116	0.110
PS—9800	0.1	2920	0.417	2.09	1.092	0.088
	1.0	2440	0.500	1.91	1.108	0.103
PS—35 000	0.1	8110	0.150	3.80	1.005	0.005
	1.0	3940	0.310	2.65	1.030	0.030
PS—110 000	0.1	10170	0.120	4.64	1.023	0.023
	1.0	3240	0.377	2.62	1.048	0.047
PS—200 000	0.1	9120	0.134	4.61	1.024	0.024
	1.0	2890	0.423	2.60	1.051	0.050
PS—470 000	0.1	5350	0.228	3.81	1.046	0.045
	1.0	1910	0.639	2.27	1.083	0.080
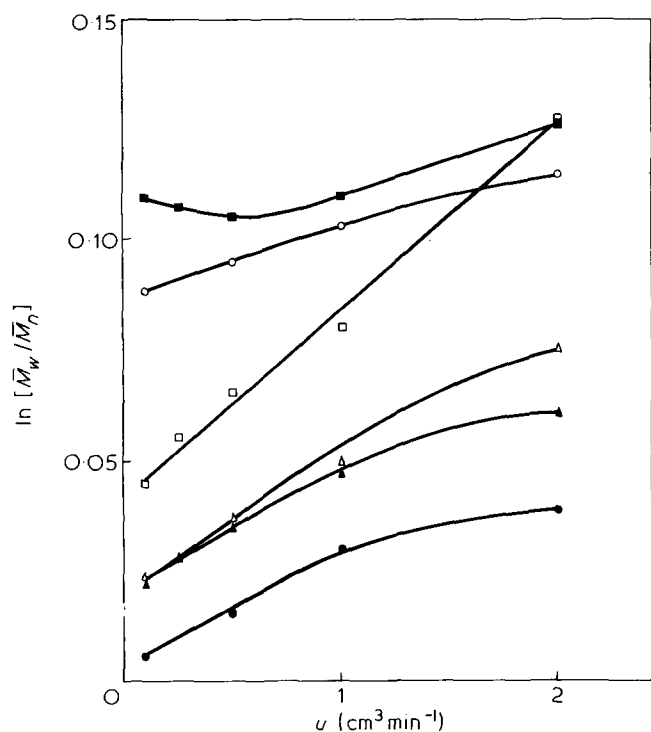
Figure 5 Semilogarithmic plot of polydispersity *versus* eluent flow rate for polystyrene standards. Symbols as in *Figure 4*
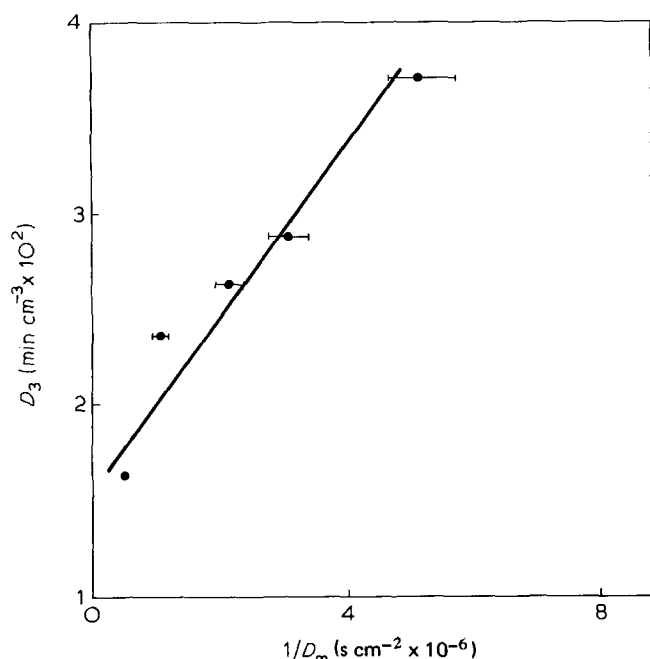

Figure 6 Dependence of the slope of the plot of log(polydispersity) *versus* flow rate in *Figure 5* on the reciprocal of the diffusion coefficient of polystyrene

particles ($d_p \sim 40 \mu$m) that H varies little with flow rate for a non-permeating polystyrene having a molecular weight of 160 000. The same polystyrene was also examined with a column containing non-porous glass particles ($d_p \sim 120 \mu$m) and again H was independent of flow rate[33]. The results of Kelley and Billmeyer[33] also suggest that H remains constant as flow rate increases for polystyrenes with molecular weights 19 800 and 97 200 and that H only falls at lower flow rates for low molecular weight solutes ($\leqslant 10\,300$). Giddings, Bowman and Myers[34] also observed that H hardly changed for a non-permeating polystyrene (molecular weight =

20 400) with a column of glass particles ($d_p \sim 55 \mu$m). We have also shown that non-permeating polystyrenes with molecular weights 111 000, 402 000 and 470 000 have H values which exhibit little change with flow rate for silica particles ($d_p \sim 20$, 44 and 65 μm)[35,36], alumina particles ($d_p \sim 20 \mu$m)[36], and crosslinked polystyrene particles ($d_p \sim 20 \mu$m)[5].

Therefore, it is reasonable to assume that term I in equation (9) may be regarded as a constant, independent of flow rate. Furthermore, for one standard, $[\bar{M}_w/\bar{M}_n]_T$ will be constant. A minimum in the efficiency *versus* flow rate plot occurs because term II in equation (3) and (9) is larger than term III at low flow rates. As the molecular weight of a polymer increases, the value of D_m will decrease so that term II decreases and term III increases. Therefore, term II becomes larger than term III at much lower flow rates as solute size increases (see *Figures 4* and *5*). From the observation in *Figure 5* that a minimum occurs at a flow rate of 0.5 cm³ min⁻¹ for PS-3600 and that a minimum is not observed for the other standards of higher molecular weight, we shall neglect term II in equations (3) and (9) for polystyrenes with molecular weights above 10 000 at practical flow rates. The slope D_3 in *Figure 5* was determined for the linear part of each curve at flow rates $\leqslant 1.0$ cm³ min⁻¹ for all polystyrene standards except PS-3600, and the values are plotted *versus* $1/D_m$ in *Figure 6*. Values of D_m for the polystyrene standards in tetrahydrofuran at 293K were estimated with the Wilke–Chang equation[37,38]. Error bars are shown for values of D_m as the average error in D_m using the Wilke–Chang equation is 10%[38]. In order to confirm that the dependence of D_3 on $1/D_m$ is not greatly influenced by R, the data in *Figure 6* are plotted in *Figure 7* according to term
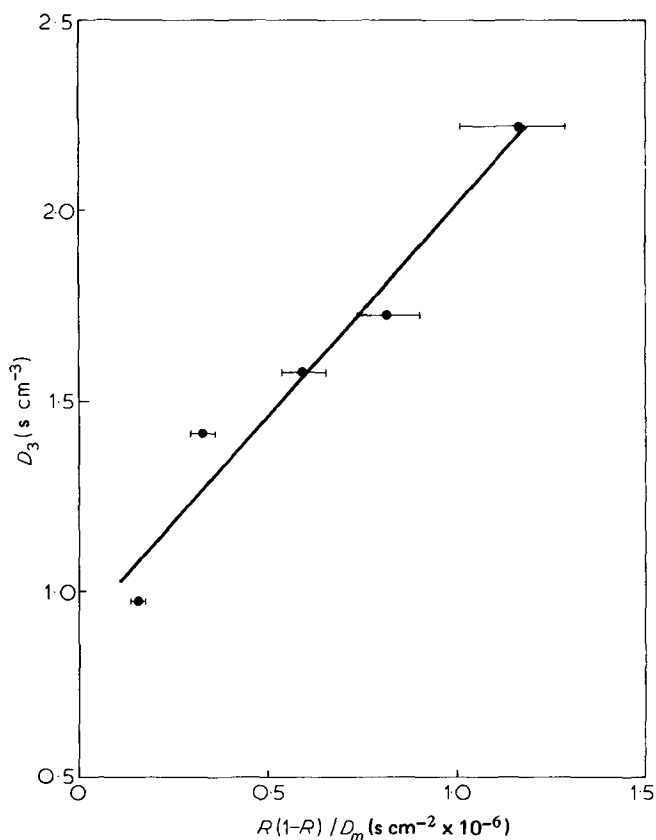

Figure 7 Dependence of the slope of the plot of log(polydispersity) *versus* flow rate in *Figure 5* on the mass transfer term in equation (9)
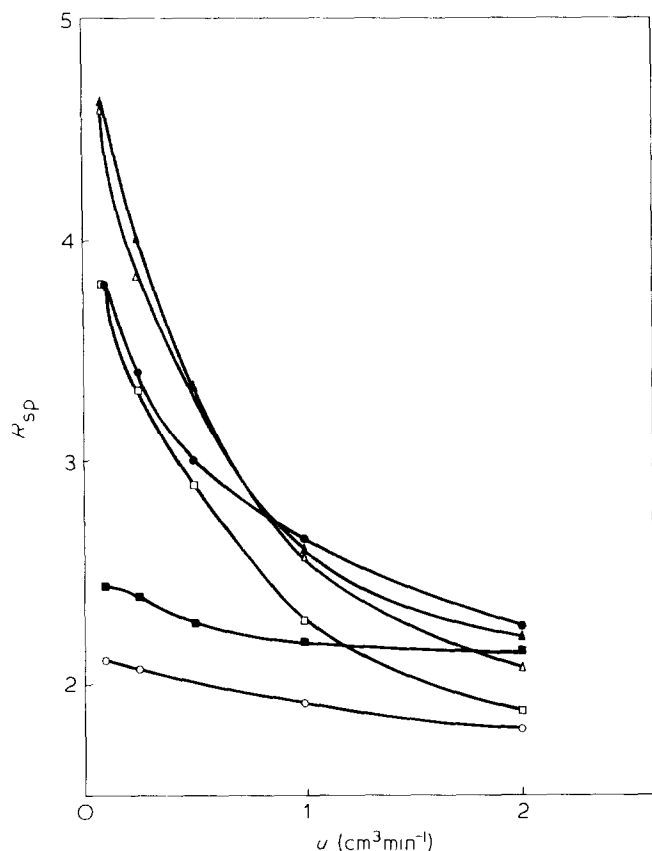
Figure 8 Dependence of specific resolution from equation (10) on eluent flow rate for polystyrene standards. Symbols as in *Figure 4*

III in equation (9). The value of V_0 for the calibration curve in *Figure 3* was required for calculating values of R and was assumed to be 45% after considering the exclusion limits from the calibration curves in *Figure 2*. It is concluded that larger molecules will have higher mass transfer dispersion because of lower values of D_m.

The plots in *Figures 5, 6* and *7* confirm that equation (9) is a reasonable representation of the dependence of the experimental polydispersity on eluent flow rate for polymers with molecular weights $> 10^4$. From *Figures 6* and *7* we may conclude that term III which results from dispersion due to mass transfer is the major contributor to chromatogram broadening for high polymers at fast flow rates. In *Figures 4* and *5*, it may be observed for several high molecular weight standards that the curves tend to flatten as u increases. Such a tendency[26] may be accounted for by the Giddings coupling theory[6] for H or by the theory proposed by Kelley and Billmeyer[39]. Therefore, equation (9) may only be applicable over a limited range of flow rate but the terms in the equations arising from the more rigorous theories are less easy to evaluate. It is evidenced in both *Figures 4* and *5* that term II cannot be neglected for low polymers.

The results in *Figures 4* and *5* have important consequences for experimental high speed and high resolution g.p.c. separations. For low polymers, the flow rate may be chosen to give the optimum column efficiency. At slower flow rates chromatogram broadening will increase because of term II in equation (3). At faster flow rates the mass transfer term will not be too significant for low polymers with high D_m, and so high speed separations may be performed with little loss in efficiency. Examples of high resolution separations of low polymers are reviewed elsewhere [40]. For high polymers the loss in column efficiency at fast flow rates is pro-

nounced because of the magnitude of the mass transfer term. Consequently, the most efficient separations of high polymers will be performed at low flow rates.

The large increase in chromatogram broadening for high polymers at fast flow rates and the much smaller change for low polymers is also demonstrated qualitatively in a plot of specific resolution R_{sp} *versus* flow rate in *Figure 8*. Specific resolution was calculated with the relation given by Yau and coworkers[12]:

$$R_{sp} = \frac{0.576}{D_2 \sigma} \tag{10}$$

where D_2 is the slope of the linear calibration curve of log(molecular weight) *versus* V and σ is the standard deviation for the chromatogram of a standard having a narrow molecular weight distribution. We have assumed a constant value of D_2 which was calculated by least squares analysis of the data for molecular weights $\leqslant 470\,000$ in *Figure 3*. A quantitative interpretation of R_{sp} for the non-linear calibration in *Figure 3* would require the dependence of D_2 on molecular weight and the variation of polydispersity among the polystyrene standards. The parameter R_{sp} is a useful means of comparing the experimental performance of different columns and of the same columns in different laboratories. Our values of R_{sp} in *Figure 8* at a flow rate of $2.0\ cm^3\ min^{-1}$ indicate a better performance than that for the same length of μStyragel columns reported by Yau and coworkers[12] who obtained $R_{sp} = 1.14$ for a polystyrene standard with a molecular weight of 97 200. Typical values of R_{sp} are given in *Table 1*.

Polydisperse polystyrene

Polystyrene PSGY2 was prepared under controlled conditions so that the polydispersity would be close to that calculated from the mechanism of a polymerization performed to low conversion. Values of $\bar{M}_n$, $\bar{M}_w$ and $\bar{M}_w/\bar{M}_n$ were determined from chromatograms and a summary of the molecular weight data is shown in *Table 2*. The values of $\bar{M}_n$ compare well with the value obtained by membrane osmometry. At a flow rate of $0.1\ cm^3\ min^{-1}$ the difference between the values from *HP*-g.p.c. and membrane osmometry is 5%. The values of polydispersity in *Table 2* by *HP*-g.p.c. were all smaller than the value of 1.85 which was determined for sample PSGY2 from a chromatogram obtained with g.p.c. columns (total column length = 488 cm) containing cross-linked polystyrene particles with $d_p \sim 40\ \mu m$. This experiment was performed at the Polymer Supply and Characterisation Centre, RAPRA, Shawbury, England, with an eluent flow rate of $1\ cm^3\ min^{-1}$; the polydispersity in *Table 2* at the same flow rate is much lower. The g.p.c. separation time for the chromatogram obtained at RAPRA was about 160 mir so it may be concluded from *Table 2* that the columns con-

Table 2 Average molecular weights and polydispersities for polystyrene PSGY2 by *HP*-g.p.c.

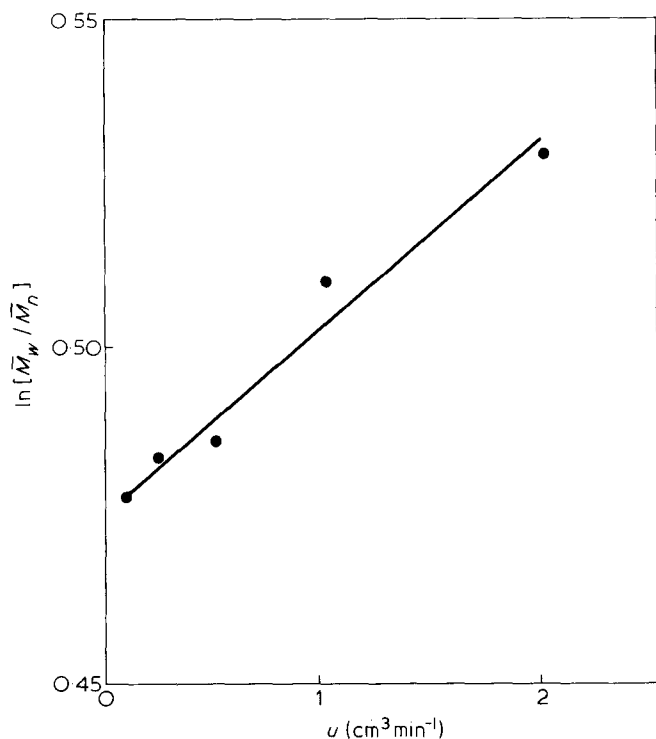| u (cm³ min⁻¹)	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
0.1	80 000	1.61
0.25	79 500	1.62
0.5	79 000	1.63
1.0	78 000	1.67
2.0	76 000	1.70

Figure 9 Semilogarithmic plot of polydispersity *versus* eluent flow rate for polydisperse polystyrene PSGY2

taining *HP*-g.p.c. microparticulate packings give faster separations (22 min for u = 2.0 cm^3 min^{-1}) and more accurate distributions than the columns containing larger particles ($d_p \sim 40 \mu$m).

A plot of the data for polystyrene PSGY2 according to equation (9) is shown in *Figure 9*. It was established in *Figures 4* and *5* that term II in equation (9) was negligible for polystyrene standards of high molecular weight ($> 10^4$) at practical flow rates. From *Figure 9* we may assume that the polydisperse polystyrene conforms well to equation (9) and that at the lowest flow rate of 0.1 cm^3 min^{-1} term IV is the major contributor to the experimental polydispersity. This second assumption is not too unreasonable because when the data in *Figure 9* are extrapolated to u = 0 the intercept is 1.609 which is very close to the value of $\bar{M}_w/\bar{M}_n$ obtained at the lowest experimental flow rate. Therefore, the experimental polydispersity determined at the flow rate of 0.1 cm^3 min^{-1} is larger than the value of 1.5 predicted from a radical chain polymerization in which termination is solely by bimolecular combination of radicals[31]. Three interpretations may be suggested. First, the difference between the values of 1.61 and 1.50 is due solely to chromatogram broadening terms in equation (9). Second, terms I and III in equation (9) can be neglected and the broader distribution results from additional termination and transfer reactions in the polymerization mechanism. The third explanation is that terms I and III are significant and that termination by combination is not the only reaction producing polymer molecules. In an attempt to distinguish these possibilities, theoretical distributions for the polymerization mechanisms in the first and second interpretations have been calculated.

The differential weight distribution $w(M)$ as a function of molecular weight for a styrene polymerization with termination solely by radical combination is given by[31]:

$$w(M) = \frac{4M^2}{\bar{M}_n^3} \exp \left[\frac{-2M}{\bar{M}_n} \right] \qquad (11)$$

This distribution may be calculated for sample PSGY2 at u = 0.1 cm^3 min^{-1} with the value of $\bar{M}_n$ determined by membrane osmometry and is compared in *Figure 10* with the distribution calculated from the experimental chromatogram by the method of Pickett, Cantow and Johnson[24]. As expected from the difference between the polydispersities, these distributions do not completely agree over all the molecular weight range.

We turn now to the second interpretation which will permit the polydispersity to exceed 1.5 when part of the termination process is by a disproportionation reaction. The differential weight distribution for a polymerization with termination solely by radical disproportionation is given by[31]:

$$w(M) = \frac{M}{\bar{M}_n^2} \exp \left[\frac{-M}{\bar{M}_n} \right] \qquad (12)$$

with a polydispersity of 2.0. For a polymerization in which both disproportionation and combination occur, we may assume that the molecular weight distribution is the sum of the weight fractions of chains produced by disproportionation and combination[31]. From equations (11) and (12) we obtain:

$$w(M) = \frac{(1 - w_2)M}{\bar{M}_{n_1}^2} \exp \left[\frac{-M}{\bar{M}_{n_1}} \right] + \frac{w_2 4M^2}{\bar{M}_{n_2}^3} \left[\frac{-2M}{\bar{M}_{n_2}} \right] \quad (13)$$

in which $\bar{M}_{n_1}$ and $\bar{M}_{n_2}$ and w_1 and w_2 are the number-average molecular weights and weight fractions for disproportionation (1) and combination (2), respectively. By considering the moments of the distribution function in terms of the moments of the two component distributions[41], it is possible to derive values for $w_2, \bar{M}_{n_1}$ and $\bar{M}_{n_2}$ in equation (13) from values for $\bar{M}_n$ and $\bar{M}_w$ calculated from the experimental chromatogram. The distribution function calculated with equation (13) did not provide a better fit than the distribution calculated with equation (11); the function from equation (13) did not improve the representation of the high molecular weight tail and predicted a weight fraction of polymer at the peak of the distribution which was too low.
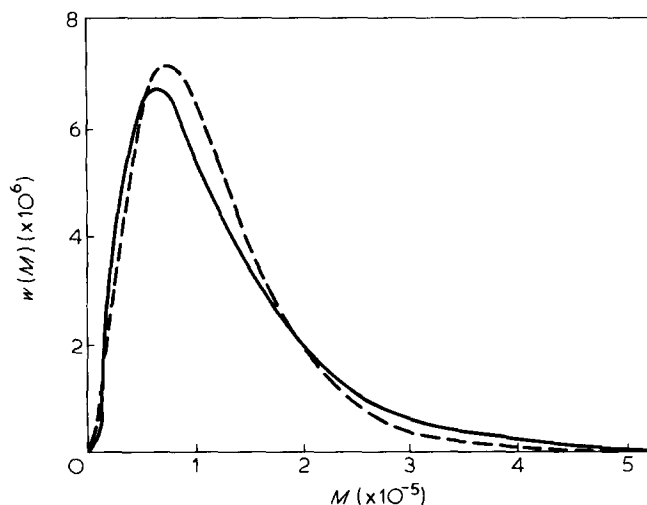

Figure 10 Differential weight distribution for polydisperse polystyrene PSGY2. ——, From experimental chromatogram determined by *HP*-g.p.c. at an eluent flow rate 0.1 cm^3 min^{-1}; — — —, from equation (11) assuming a styrene polymerization with termination solely by radical combination

It is concluded that chromatogram broadening is still significant at a flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$. The experimental distribution is broader than both theoretical distributions predicted with equation (11) (see *Figure 10*) and with equation (13). *Figures 4* and *5* demonstrate that the degree of chromatogram broadening as the flow rate increases will be more prevalent for high polymers than for low polymers. This suggests that the shape of a chromatogram of a polydisperse polymer will change as flow rate increases because of the greater dispersion effect of the high molecular weight chains. Even at a flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$ the experimental distribution in *Figure 10* is not fitted by the predicted distribution over the high molecular weight tail but is fitted at lower molecular weights ($<40\,000$). This difference between the tails of the distribution cannot be explained by our neglect of term II in equation (9). Consequently, we cannot specify an exact value of $[\bar{M}_w/\bar{M}_n]_T$ for polystyrene PSGY2. The determination of the proportions of termination by combination and by disproportionation in radical polymerization has been studied for many years. A recent comprehensive study by Berger and Meyerhoff[42,43] suggested that the fraction of termination by combination was 0.75 for styrene polymerizations at 333K. From the polydispersity of polystyrene PSGY2 studied at $0.1 \text{ cm}^3 \text{ min}^{-1}$ and the chromatogram broadening contribution, it would appear that the fraction of termination by disproportionation is much smaller than suggested by Berger and Meyerhoff. Studies of polystyrene PSGY2 with particles having $d_p \sim 5 \ \mu\text{m}$ would be desirable in order to lower chromatogram broadening even further, when it may be possible to determine precisely an accurate value of $[\bar{M}_w/\bar{M}_n]_T$ and whether a small fraction of termination by disproportionation does occur.

CONCLUSIONS

These results demonstrate that *HP*-g.p.c. with microparticulate packings in short columns will provide accurate molecular weight data when the instrumentation and operating procedures are optimized. The studies of polydispersity for polystyrene standards show that the extent of chromatogram broadening will be determined by eluent flow rate and polymer molecular weight, in agreement with a relation predicted from the theoretical consideration of solute dispersion mechanisms in chromatographic columns. High molecular weight polystyrenes are only separated efficiently at low eluent flow rates, and the decrease in column efficiency with flow rate is less significant as the molecular weight of polystyrene falls. For low polymers, efficient separations may be performed at fast eluent flow rates. Consequently, the most accurate determination of molecular weight distribution for a polydisperse polymer should be performed at a low eluent flow rate. From a comparison of experimental and theoretical distributions for the polydisperse polystyrene sample, a small chromatogram broadening contribution would appear to be present even at the lowest practical flow rate of $0.1 \text{ cm}^3 \text{ min}^{-1}$. This comparison also suggests that most of the radicals in the polymerization of styrene terminated by combination.

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