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SEPARATION BY FLOW (HYDRODYNAMIC CHROMATOGRAPHY) OF MACROMOLECULES PERFORMED IN OPEN MICROCAPILLARY TUBES

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SUMMARY

It is shown experimentally that, in principle, separations of macromolecular species are possible by differential migration in the flow of a solvent through a microcapillary tube (diameter less than *ca.* 10 μm). Compared to gel permeation chromatographic separations, hydrodynamic chromatographic separations are rapid and show little axial dispersion of the sample zones. The results are in a qualitative agreement with the theory of separation-by-flow, proposed by Guttmann and DiMarzio.

INTRODUCTION

The relatively new technique of hydrodynamic chromatography (HC), sometimes called "separation by flow", is a technique for particle separation that has attracted much interest in recent years, both in the analytical field and in technology.

Basically, particle size separation takes place when the finite dimensions of the particles are large enough to interfere with the flow profile in a packed-bed or open-tubular flow system. For geometrical reasons alone, particles are excluded from the slower moving wall regions of the flow channels and will therefore move with a velocity greater than the average velocity of the solvent, molecules of the latter being free to move over the whole width of the flow channel. Hence the larger particles elute first from the flow system, followed by the smaller ones, as is also the case in the well known polymer separation technique of gel permeation chromatography (GPC).

Experimental evidence for this effect was first reported by Pedersen[1], who separated protein mixtures in packed beds with small glass spheres. DiMarzio and Guttmann[2,3] gave the first theoretical account of the phenomenon, and it was the well known work by Small and co-workers[4,5] that demonstrated the practical feasibility of the technique in separating mixtures of monodisperse polymer latexes and colloidal dispersions of carbon blacks and silica, again using a packed-bed system with solid non-porous polymer beads. The non-porous character of the packing material ensures that, selective permeation in pores (GPC) being eliminated, separation is based solely on hydrodynamic flow principles. The present status of packed-bed HC as a high-speed analytical tool for determining the particle-size distribution of samples from molecular size to greater than 1 μm has recently been discussed by Small and Langhorst[6].

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Although the separation by a flow mechanism has also been proposed for GPC separations[2,3,7], conclusive evidence for this has never been obtained. On the other hand, separation by flow has been obtained by conducting separations of particles or macromolecules in open-tubular flow channels of reduced diameter, ensuring the absence of any GPC mechanisms. Ouano and Biesenberger[8] first published a preliminary separation of macromolecules in an open tube (diameter 1 mm), not mentioning the separation mechanism. Noel *et al.*[9] demonstrated that capillary HC is feasible in 0.4 mm tubing, separating particles in the range 0.5–30 μm in diameter (latex, pollen, silica and cells). Brough *et al.*[10] extended the useful analytical range of capillary HC down to particle diameters of 0.07 μm by applying smaller diameters of the tubing (down to 0.182 mm). Mori *et al.*[11] reported the separation of polystyrene macromolecular mixtures in a column consisting of a sintered bundle of small-diameter glass rods. This column is equivalent to a bundle of parallel microcapillary tubes with an effective hole size of about 1 μm.

It was the purpose of this work to investigate the potential of a number of individual microcapillary tubes, which are available in sizes down to about 1 μm, for HC separations. Although separations of particulate materials should also be possible in these microcapillaries, we first concentrated on the separation of macromolecules in order to compare the results with those obtained by the widely used GPC technique.

In LC the column diameter must be as small as possible to limit the width of the sample zones. In HC, the same advantage of limited zone dispersion can be obtained by using microcapillaries. We expect, therefore, that the separations obtained so far in wide-bore capillaries, *e.g.*, by Noel *et al.*[9], and Brough *et al.*[10], will be greatly improved by the use of microcapillary tubes.

EXPERIMENTAL

Experimental confirmation of the foregoing is possible, using the same apparatus as in our microcapillary LC work[12]. This apparatus has been specially designed so as to ensure that peak broadening outside the column is carefully minimized. The method of sample injection involved a split technique which, at sufficiently high splitting ratios of about $1:10^3$, keeps sample dispersion within practical limits.

It is possible[12] to reduce peak dispersion in the flow-through cells of common LC UV absorption detectors by adding scavenger ("make-up") liquid to the column effluent. However, this results in considerable dilution of the sample concentrations, to such an extent that in columns more than 10 μm in diameter only major components of the sample can be detected. For columns less than 10 μm in diameter, as used in HC, the dilution by scavenger liquid is unacceptable and alternative detection methods must be considered.

In this work we examined the possibility of "on-column detection", using the end section of the (quartz) column itself as the detector cell[13]. In this way additional peak broadening in connections and in the flow cell of the detector is avoided. Using fused silica columns, which have recently become available from SGE (North Melbourne, Australia), it is then possible to detect UV-absorbing substances such as polystyrenes (PS) in a suitable solvent by measuring the transmitted radiation per-

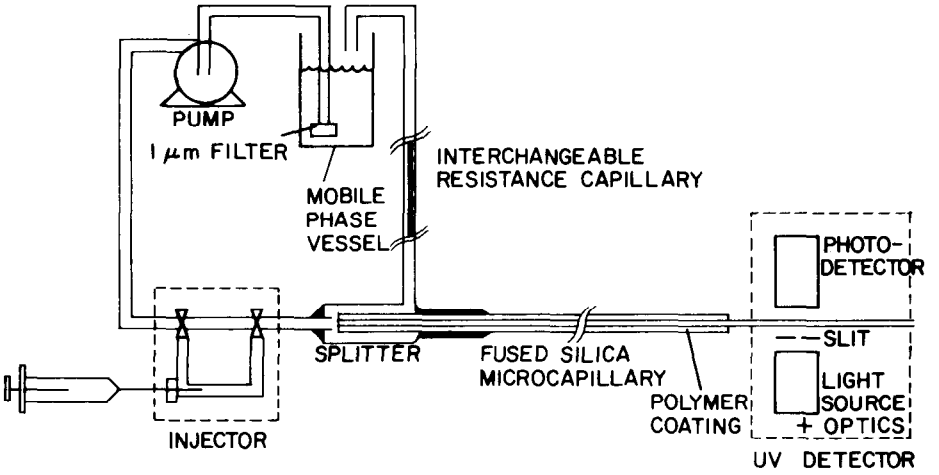

Fig. 1. Schematic representation of the apparatus for microcapillary hydrodynamic chromatography. Pump, injector and detector are standard HPLC instruments. The end section of the quartz microcapillary (polymer coating removed by burning off) is used as the detector cell itself.

pendicular to the column. A drawback of this method is the extremely short path length of the measuring cell, which is roughly the same as the column radius. In this work we used two columns with nominal I.D. of 10 and 1.2 μm (measured with a microscope).

Fig. 1 shows the experimental arrangement. The injector is a Waters U6K and the pump a Waters M6000. The slit width in the UV detector (Jasco Uvidec II) has been modified and is so small that most of the radiation only passes through the liquid stream in the centre of the microcapillary. However, stray light via refraction through the relatively thick quartz wall of the column could not be avoided completely.

Although non-ideal in many respects, the detector described was sufficiently sensitive to detect several PS samples (Waters standard polymer samples) in tetrahydrofuran (THF), the mobile phase solvent used.

RESULTS AND DISCUSSION

Typical separations obtained with the above apparatus are shown in Figs. 2 and 3 for the columns of I.D. 10 and 1.2 μm, respectively.

The successful HC operation of both columns allows two conclusions to be drawn: (a) separation by flow for macromolecular PS molecules can indeed be obtained in microcapillary tubes; and (b) on-column detection is feasible, even for these small-sized tubes. Previously the minimum tube diameter that has been used with an on-column detection technique has been 40 μm[13].

In all our experiments detection proved to be the most problematical step. Positioning of the column end section with respect to the light beam and the slit was critical.

For any signal to be obtained, sample concentrations had to be high [*ca.* 1 % (w/w)], which implies that the sample solutions were viscous. The residence time

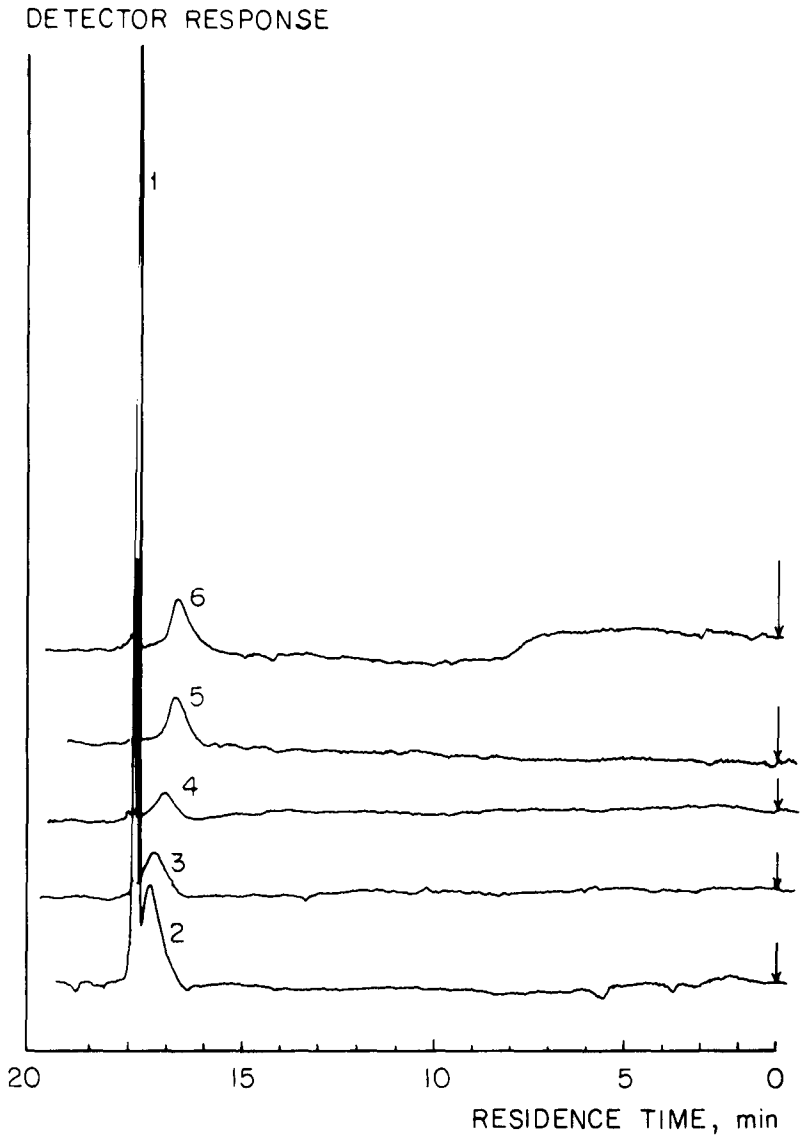

Fig. 2. Hydrodynamic chromatography of polystyrenes in a 10-μm microcapillary column. Column length 450 cm. Solutes: 1 = toluene (t_M = 985 sec); 2 = PS $2.7 \cdot 10^6$; 3 = PS $3.7 \cdot 10^6$; 4 = PS $5.5 \cdot 10^6$; 5 = PS $6.8 \cdot 10^6$; 6 = PS $8.4 \cdot 10^6$. Mobile phase, THF. Detection at 212 nm, 0.01 a.u.f.s.

behaviour of the PS molecules is influenced by this in two ways. First, a viscous sample is not easy to inject and long injection times (up to 10 sec) are required. This leads to additional residence time and peak dispersion. Second, it is well known[14–16] that in these concentrated polymer solutions the sizes of the macromolecules are less than in dilute solutions. This, of course, leads to increased retention times in HC. In particular, within a sample zone the parts of higher concentration migrate more

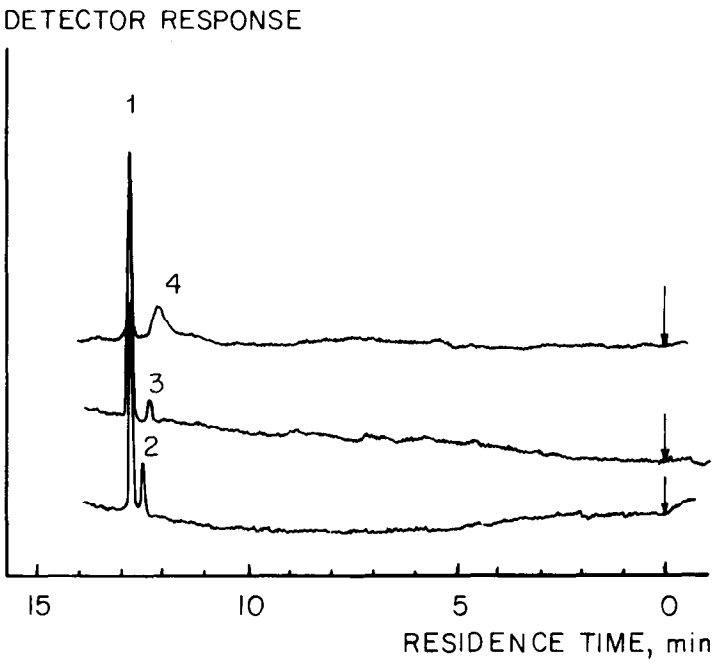

Fig. 3. Hydrodynamic chromatography of polystyrenes in a 1.2-μm microcapillary column. Column length 126 cm. The original chromatogram for PS 670,000 was produced in such a way that t_M = 1278 sec; for easy comparison, the upper trace in the figure is a recalculated reproduction where toluene (1) elutes at the same time as in the other traces. Solutes: 1 = toluene (t_M = 763 sec); 2 = PS 51,000; 3 = PS 160,000; 4 = PS 670,000. Mobile phase, THF. Detection at 220 nm, 0.005 a.u.f.s.

slowly than the diluted front and rear parts. This may be the mechanism behind the typical fronting peak shapes often obtained (*cf.*, Figs. 2 and 3).

Although we are certain that residence times are influenced by both of the concentration effects mentioned, we nonetheless tried to match the residence times observed with those predicted by the DiMarzio and Guttmann theory, the latter being valid for infinite dilution only. To this end we used effective sizes (hydrodynamic radii) of the PS molecules as found in light scattering experiments by Mandema and Zeldenrust[17], also valid for infinite dilution only. Although the hydrodynamic radius of polymer molecules differs slightly from the effective size of these molecules as defined by Van Kreveld and Van den Hoed[18], the approximation is considered to be sufficiently good for the present, qualitative purposes.

Fig. 4 shows that at least a qualitative agreement exists between our experiments and the theory. More quantitative data can only be obtained if the detection is improved in such a way that sufficiently high signal-to-noise ratios can also be obtained for dilute samples.

At all events, the present results indicate that HC of macromolecular species is indeed possible and that it has potential for development into a useful method for the determination of molecular weight distributions. Compared with GPC, HC is rapid and probably requires no corrections for the very limited peak dispersion for most samples with a relatively broad molecular weight distribution (MWD) encountered in

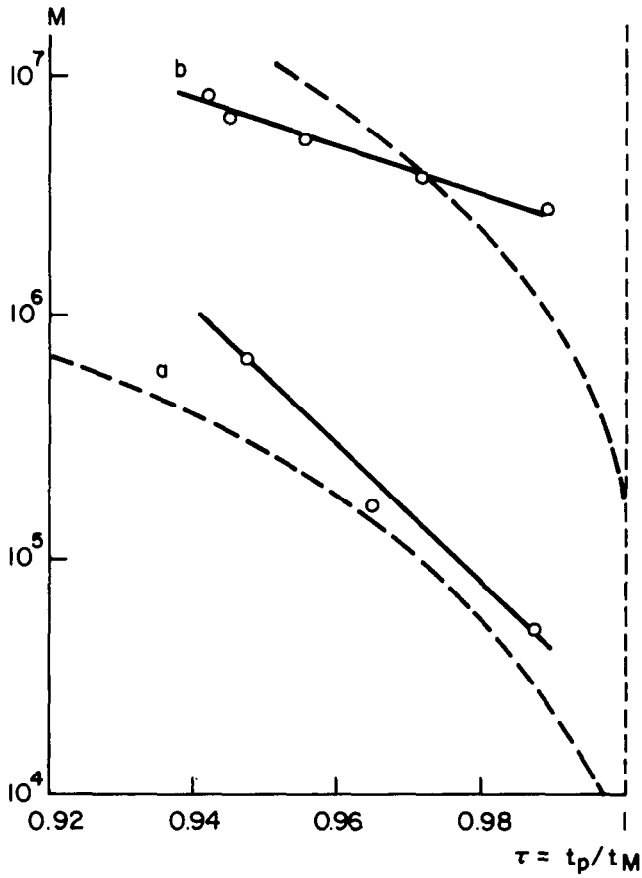

Fig. 4. Comparison of experimental residence times with those predicted by the DiMarzio and Guttmann theory[2,3]. τ is the relative residence time [polymer residence time t_p over t_M, the residence time of a small molecule (such as toluene or the mobile phase solvent)]. O—O, Experimental points; – – –, theory. (a) Column I.D. = 1.2 μm, column length L = 126 cm, t_M = 763 sec; (b) I.D. = 10 μm, L = 450 cm, t_M = 985 sec.

practice. This is an important advantage of HC over GPC, as the axial dispersion corrections in GPC are very tedious[19].

The limited extent of peak broadening in HC can be observed in the present experiments and is also predicted by the DiMarzio and Guttmann theory. In particular for the 1.2 μm I.D. column (Fig. 3) the peak widths of small molecules such as toluene and of the PS samples are very similar and of the order of 10 sec (at their base) only.

If we try to match the peak broadening observed for small molecules with existing theories[20,21], we find that, in all our experiments, the peak widths are larger than the predicted values, although of the correct order of magnitude. Typical examples of this, for both columns, are collected in Table I. The first striking feature is that the peak width for small molecules such as toluene is roughly double the width expected on the basis of the Taylor–Aris theory, which predicts that σ =

TABLE I

COMPARISON OF EXPERIMENTAL AND THEORETICAL PEAK WIDTHS
In terms of the peak standard deviation (σ) in seconds.

Solute	*1.2 μm column*		*10 μm column*	
	σ_{exp}	σ_{theor}*	σ_{exp}	σ_{theor}*
Toluene	2.25	1.26	1.50	0.80
PS 51,000	2.25	0.41	–	–
PS $3.7 \cdot 10^6$	–	–	18.0	11.13

* Numerical values used in the calculation were as follows: $\mathscr{D}_M$ (toluene/THF) = $2.66 \cdot 10^{-5}$ cm²/sec (ref. 22); $\mathscr{D}_M$ (PS 51,000/THF) = $7.63 \cdot 10^{-7}$ cm²/sec (ref. 17); $\mathscr{D}_M$ (PS $3.7 \cdot 10^6$/THF) = $6.81 \cdot 10^{-8}$ cm²/sec (ref. 17).

$[(R^2 t_M/24\mathscr{D}_M) + (2\mathscr{D}_M t_M{}^3/L^2)]^{1/2}$ (refs. 20 and 21), where (R is the column radius, t_M the mean residence time, $\mathscr{D}_M$ the molecular diffusion coefficient and L the column length). This equation was derived for peak broadening by convective dispersion (first term on the righ-hand side) and axial molecular diffusion (second term on the right-hand side) in the column itself.

The conclusion we draw from this is that extra-column broadening effects play an important role in the total peak width observed. This is in accordance with our earlier observations in microcapillary LC[12]. In the present instance, where shorter and narrower columns are used than in LC, extra-column broadening from injection and detection is even more critical. With the on-column detection method used, a contribution to peak broadening can be found in the finite length of the column end section which is used as the measuring cell. In these experiments the cell length was about 0.5 cm, which is a substantial part of the zone length occupied by the samples which pass through the "cell". For example, the toluene peak in the 1 μm column occupies approximately 2.2 cm in the column (corresponding to $6\sigma_{exp}$, the total peak width at the base in time units) and in the 10 μm column 4.1 cm. In both instances the length of 0.5 cm is certainly long enough to contribute to the peak broadening observed.

For polymer samples with a very narrow MWD, as used in this work, peak broadening in the columns may be found from the residence time distribution as predicted by the DiMarzio and Guttmann theory[2,3]. Table I shows that, in both columns used, the peak widths observed are substantially larger than those predicted, although still of the correct order of magnitude. The additional peak broadening arises from several sources. First, of course, the extra-column broadening as found for toluene also applies to the polymer samples. Second, viscosity and concentration effects as mentioned before are present and will also contribute to the observed peak widths.

A quantitative test of the DiMarzio and Guttmann dispersion theory will only be possible if we are able to avoid concentration effects (by lowering detection limits) and to correct for extra-column broadening effects. Once this has been accomplished, and if the DiMarzio and Guttmann theory is indeed confirmed, HC will have a promising future as a rapid means of determining MWDs of polymer samples, pro-

vided, of course, that the construction of HC apparatus suitable for practical application proves feasible.

From the theory we have been able to estimate the peak capacity (N) of HC chromatograms [*i.e.*, the number of completely resolved peaks that can be accommodated between the minimum ($\frac{1}{2}t_M$) and maximum (t_M) residence times]. The result is of the form $N \propto t^{1/2}R^{-3/2}$ and indicates that, in sufficiently small diameter tubing (of the order of 1 μm), and with sufficiently long (but in an absolute sense short) times (of the order of several minutes), peak capacities of 50–500 will be possible. This would indeed allow the rapid determination of MWDs.

CONCLUSIONS

Separation by flow in microcapillaries can be realized experimentally in fused silica microcapillaries using an on-column detection technique.

The residence times and peak widths measured in experiments on polystyrene samples correspond qualitatively to the theory put forward by DiMarzio and Guttmann.

Quantitative agreement between experiment and theory can only be investigated if the detection is substantially improved. Concentration effects are at present an obstacle to more quantitative data being obtained.

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