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BAND DISPERSION IN HIGH-PERFORMANCE EXCLUSION CHROMATOGRAPHY

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SUMMARY

The dispersion of polymer samples eluted in the exclusion mode from columns of Hypersil and SG60F (a highly porous silica gel) has been measured for a range of polymer standards over a range of elution velocities and from columns of different lengths. From experiments with columns of different lengths operated at the same velocity, the polydispersities of the polymer standards were obtained and ranged from 1.007 to 1.052. From experiments at different linear velocities, mass transfer coefficients were obtained from which the ratio of the diffusion rates of polymers in bulk eluent to the diffusion rates within the silica gel matrix could be found. For Hypersil these ranged from *ca.* 6 for PS2K and PS4K (MW 2000 and 4000 respectively) to *ca.* 17 for PS33K (MW 33,000) indicating fairly severe restrictions to diffusion within the pores of the matrix especially for nearly excluded materials.

Measurements with a pure oligomer of polystyrene (MW \approx 3000) confirmed the validity of the above method of separating spreading effects of polydispersity and kinetics and gave a reduced plate height-velocity curve overlapping that for benzene.

Measurements of spreading of excluded polymer samples indicated anomalous behaviour at low flow-rates and with long columns.

Measurements of the loading capacity in exclusion chromatography showed that samples should not exceed *ca.* 1 μg per cm^3 of column packing if overloading is to be avoided.

It is concluded that the dispersion of bands of polymers eluted in the exclusion mode from silica gels obeys the same kinetic relationships as the dispersion of bands in retentive chromatography. The study confirms, however, that the major contributor to the dispersion of polymer samples eluted in the exclusion mode is their polydispersity.

INTRODUCTION

Despite much experimental work there is still no universal consensus as to the dominant exclusion mechanism for polymers in porous matrices, except that the exclusion is determined largely by molecular size, the largest molecules being the

most excluded. Formally the degree of exclusion is represented by the parameter K in eqn. 1 or the parameter k'' in eqn. 2.

$$V_R = V_0 + KV_p \quad (1)$$

$$V_R = V_0(1 + k'') \quad (2)$$

where V_R is the elution volume of the polymer, V_0 is the volume of the space outside the particles of column packing and V_p is the pore volume within the particles themselves. When the pore diameter is much smaller than the particle diameter V_0 and V_p are well defined. While the parameter K is most useful when correlating experimental data with predictions from geometrical models, k'' is the relevant parameter to use when considering the kinetics of band dispersion in exclusion chromatography.

Various models have been proposed for explaining the degree of exclusion of polymers from gel networks. Porath and Flodin¹ originated the purely geometric model which proposes that the exclusion is entirely determined by the restriction that the centre of mass of a polymer molecule cannot approach a pore wall more closely than the radius of the molecule which Porath and Flodin assumed to be spherical. Cassassa^{2,3} carried out a rigorous mathematical analysis for random-chain polymers in voids of various shapes but eventually concluded that exclusion curves predicted using a hard-sphere model of the polymer differed insignificantly from those predicted by the random chain model if the correct hydrodynamic radius was used. Giddings *et al.*⁴ considered the exclusion of molecules of other shapes than spherical.

More recently Van Krefeld and Van den Hoed⁵ used a random sphere model of the gel to calculate the exclusion curve for spherical polymer molecules and obtained almost exact agreement between their theoretical predictions and experimental data obtained with polystyrene standards chromatographed on Porasil, a spherical silica gel. There seems little question then that purely geometrical or statistical models can explain existing data at least for exclusion of polymers by rigid gels.

Other theories have, however, been proposed, in particular the so-called restricted diffusion theory. In the model of Ackers⁶ it is proposed that the rate of diffusion of polymers is slowed down by the constriction of the pores and the consequent friction when polymers approach the walls. This prevents full equilibration of the polymer between the region outside the particles and the pore region inside, so that the largest molecules on average migrate through the bed faster than the smaller molecules which have time fully to explore the pores. Yau and Malone⁷ developed this model further and derived an expression for K which included the diffusion coefficient of the polymer and the flow velocity. However this theory predicts that at low flow-rates all K values should tend towards unity which is at variance with experiment. In their later work^{8,9} they therefore combined their restricted diffusion theory with the geometric theory to avoid this inconsistency.

In assessing the validity of the restricted diffusion model it is relevant to consider the near-equilibrium theory of chromatography developed by Giddings¹⁰. Giddings shows in a rigorous manner that band spreading in chromatography is a direct consequence of slow equilibration between the mobile zone (outside the

particles and having a volume V_0) and the static zone (inside the particles and having a volume V_p). Indeed, the plate height H is directly proportional to the time constant for equilibration, τ , provided only that τ is much smaller than the overall elution time. If the equilibration time τ is so long that some molecules never explore their statistical fraction of the pore volume then the theory predicts that the peaks obtained will be wide and asymmetrical. Since we always desire narrow peaks in chromatography, it is clear that all effective high-performance exclusion chromatography must be carried out under near-equilibrium conditions when equilibration is rapid compared to elution, and that under these conditions only thermodynamic factors can control the degree of exclusion. Further evidence against the restricted diffusion theory comes from the failure of its prediction that the elution time should be a function of flow velocity. At least for porous silica microspheres elution times under normal experimental conditions are independent of elution velocity¹¹.

We therefore take the view, supported by Cassassa¹², that the near-equilibrium theory of Giddings¹⁰ and hence the standard kinetic equations for chromatography governing plate height should apply equally to exclusion and retentive chromatography. It was the aim of this work to examine this hypothesis by further definitive experiments.

According to chromatographic theory the reduced plate height, h ($h = H/d_p$, where H = plate height, d_p = particle diameter) should depend upon the reduced velocity v ($v = ud_p/D_m$ where u = linear eluent velocity, D_m = diffusion coefficient of solute in eluent) according to well known relationships of the Van Deemter type¹³. In this work we have used uniform porous spherical particles of silica gel and can therefore use eqn. 3 for the reduced plate height arising from kinetic processes in a bed of porous spheres¹⁰:

$$h_{\text{kin}} = \frac{2\gamma}{v} + Av^{\frac{1}{2}} + \frac{1}{30} \frac{k''}{(1+k'')^2} \frac{D_m}{D_s} v \quad (3a)$$

$$= B/v + Av^{\frac{1}{2}} + Cv \quad (3b)$$

In eqn. 3 the first term gives the contribution from axial molecular diffusion, γ being the obstructive factor for molecular diffusion in the bed as a whole. The second term is largely empirical¹³ and is the contribution from the complex pattern of flow in the mobile zone. The third term represents the non-equilibrium contribution arising from slow mass transfer within the static zone, that is within the pores of the gel. D_m is the diffusion coefficient of solute in bulk eluent while D_s is the mean diffusion coefficient of solute within the gel which for this purpose is considered to approximate to a homogeneous sphere. k'' , the zone capacity ratio, according to the equilibrium theory is given^{10,14} by:

$$k'' = \frac{\text{quantity of solute in stationary zone}}{\text{quantity of solute in mobile zone}} \quad (4)$$

From eqns. 1 and 2 k'' is simply related to K , the fraction of the pore volume accessible to the solute, by:

$$k'' = K(V_p/V_0) \quad (5)$$

It was the purpose of this work to demonstrate that the standard kinetic equations for retentive chromatography applied equally to exclusion chromatography in a rigid gel.

Fig. 1 shows a typical chromatogram of polystyrene standards (partially or completely excluded), benzene (fully permeating) and two retained solutes on a small pore silica gel. It illustrates the continuity of exclusion and retentive chromatography. It may be noted that the peaks for the polymers PS4K and PS20K are significantly wider than that of benzene even though they elute earlier. This is a common observation in exclusion chromatography. Does it arise because peak dispersion in exclusion chromatography obeys different rules to peak dispersion in retentive chromatography, or is it explained by the low diffusion coefficients of polymers which make the reduced velocities and hence plate heights for their elution very high, or is it due to the inevitable polydispersity of the samples themselves?

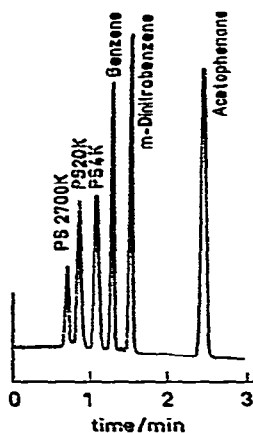


Fig. 1. Chromatogram of excluded and retained solutes on Hypersil. Column, 101 × 7 mm; eluent, dichloromethane; detector, UV at 254 nm.

Knox and McLennan¹⁵ showed that some previous formulae to correct the plate height for the polydispersity, P , of a polymer sample were seriously in error, and that the true formula for combining the dispersion due to kinetic effects with that arising from polydispersity was given by

$$h_{app} = h_{kin} + h_{poly} \quad (6a)$$

$$= h_{kin} + (L/d_p) (P-1) (1+\alpha) (S/V_R)^2 \quad (6b)$$

where h_{app} is the apparent reduced plate height measured from the second moment of the eluted peak, and h_{kin} is the kinetic contribution given by eqn. 3 in our case. In the second term arising from the polydispersity, L is the column length; α is a weak function of $(P-1)$ having a value of 0.1 when $P = 1.03$; and S is the negative inverse slope of the calibration curve for the exclusion material namely $-dV_R/d \ln M$ (where M is the molecular weight of the solute).

Knox and McLennan showed that unless P was well below 1.01, h_{app} would be dominated by the contribution from polydispersity in columns of even moderate plate efficiency, and therefore that special measures would be required to isolate h_{kin} and determine it accurately. The study described below shows, in fact, that the main cause of the spread of the peaks for the polymers in Fig. 1 is indeed their polydispersity which we show to be 1.031 for PS4K and 1.013 for PS20K.

Eqn. 6 taken with eqn. 3 enables h_{kin} and P to be independently determined, for if a series of columns of different lengths, L , are packed equally well, h_{kin} will remain constant while the contribution from the polydispersity of the sample will be proportional to L . The polydispersity is obtained from the gradient of the plot of h_{app} against L . The intercept of the line gives h_{kin} . Generally h_{kin} will be small and the accuracy of determination low. However, h_{kin} depends upon eluent velocity while the polydispersity contribution is independent of velocity. Thus the velocity dependence of h_{kin} is obtainable if a fairly short column is used and the flow-rate varied. Since D_m is low for polymers, the values of v at which separations are likely to be carried out will be high. It then follows that the mass transfer term will dominate h_{kin} and that the velocity dependence should provide data on the magnitude of C (eqn. 3b). This is fortunately the most important of the plate height parameters since it relates to the mass transfer mechanism, the efficiency of which determines whether a near-equilibrium theory or a restricted diffusion theory will hold in exclusion chromatography.

Finally, it should be possible to fractionate a polymer standard in order to isolate either a narrower cut of lower polydispersity using exclusion chromatography or even a single oligomer by using adsorption chromatography.

EXPERIMENTAL

The equipment consisted of the conventional units: solvent reservoir, column and injector, UV detector and recorder.

For high flow-rates an Orlita DMP-AE 10.4 pump was used (Orlita, Giessen, G.F.R.). For low flow-rates and pressures below 50 p.s.i. eluent was delivered from a thick-walled bottle pressurized by nitrogen (for details see Knox and Parcher¹⁶). A mercury manometer measured the pressure. Columns were made in-house from 7-mm bore Apollo liquid chromatography tubing (Magnus Scientific, Sandbach, Great Britain). Tubes from 50 to 500 mm long were vacuum gold-brazed into fittings made to couple to Shandon pattern injectors (Shandon, Runcorn, Great Britain). The detector was a CE 212 photometer (Cecil, Cambridge, Great Britain) containing an 8- μ l flow cell. Experiments in which the injector was connected to the detector via a "zero-length column" showed that the volume measured across the base of the peak produced by the injector and detector alone was 30–50 μ l. This was sufficiently small to have no significant effect on the width of peaks from totally excluded solutes eluted from the shortest column used.

Two packing materials were used in the work, Hypersil (Shandon) and an experimental batch of wide pore silica gel denoted SG60F kindly gifted by the Materials Preparation Unit, (AERE, Harwell, Great Britain). The physical properties of the two materials are given in Table I.

Columns were slurry-packed using a 60-cm³ displacement pressure intensifier

TABLE I
PHYSICAL PROPERTIES OF COLUMN PACKINGS

Packing	Mean particle diameter (μm)	Surface area ($\text{m}^2 \text{g}^{-1}$)	Pore volume ($\text{cm}^3 \text{g}^{-1}$)	Mean pore diameter (\AA)
Hypersil	6*	200***	0.70**	120**
SG60F	7.5***	100***	1.76***	600***

* Measured from electron micrographs.

** Calculated from Hg porosimetry, pore volume confirmed by elution data.

*** Quoted by supplier.

(Haskel, Burbank, Calif., U.S.A.) with methanol as the dispersing liquid. Columns were packed upwards and at least 100 cm^3 of pure methanol was passed in addition to the volume of the slurry. Subsequent to the passage of the methanol, the system was allowed to depressurize slowly before disconnecting the column, preparing the column top, fitting the top gauze and topping up with glass beads into which injections were made.

Before use with polymer standards, columns were tested by elution of small samples of benzene using dichloromethane as eluent. Columns were accepted if the reduced plate height did not exceed 4, it being normally around 2 for a 100 mm column.

Polystyrene standards were obtained from Waters Assoc. (Northwich, Great Britain) and their properties as given by the suppliers are listed in Table II. The diffusion coefficient for benzene in dichloromethane was obtained from the Wilke-Chang equation¹⁷, while those for the polystyrenes are based upon those used by Van Krefeld and Van den Hoed¹⁸ which are in good agreement with those of Hendrickson¹⁹. The values have been increased by 25% to take account of the

TABLE II
PHYSICAL PROPERTIES OF POLYMER STANDARDS

Sample	Relative molecular weight*			$10^{12} \times D_m (\text{m}^2 \text{sec}^{-1})$
	M	M_w	M_n	
Benzene	78	—	—	3080**
PS2K	—	2100	1950	630***
PS3K	2900	—	—	490
PS4K	—	4000	3100	410
PS10K	—	10,000	9600	240
PS20K	—	20,800	20,200	160
PS33K	—	33,000	36,000	115
PS11K	—	111,000	111,000	55
PS200K	—	200,000	193,000	40
PS470K	470,000	—	—	24
PS2700K	2,700,000	—	—	8

* Stated by Waters Assoc.

** Calculated for dichloromethane by Wilke-Chang equation.

*** See text.

viscosity change in going from tetrahydrofuran (viscosity = 5.5×10^{-4} N sec m^{-2}) to dichloromethane. They are given by

$$D_m/m^2 \text{ sec}^{-1} = 6.0 \times 10^{-8} M^{0.60} \quad (7)$$

where M is the relative molecular weight of the polymer.

The dichloromethane used throughout was HPLC grade supplied by Rathburn Chemicals (Walkerburn, Great Britain). Typical injections made by microsyringe were 1–5 μ l of 0.1% solutions of the solutes in dichloromethane.

RESULTS AND DISCUSSION

(A) Calibration curves and structure of beds

Fig. 2 shows the molecular weight calibration curves for polystyrene standards on Hypersil and SG60F. It clearly shows the difference in the ratio V_p/V_0 between Hypersil and SG60F which was specially developed to have a very high pore volume. Using the data from Table I and an assumed density of 2200 $kg\ m^{-3}$ for silica, the relative volumes of different zones in the packings may be calculated as in Table III, where they are compared with those for other silica gels developed for exclusion chromatography.

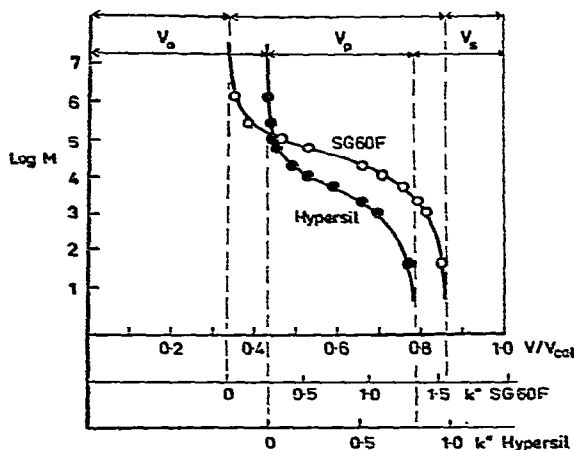


Fig. 2. Molecular weight calibration curve for polystyrene standards eluted from SG60F and Hypersil by dichloromethane. V_0 = Extraparticle void volume; V_p = pore volume; V_s = volume of silica structure; V_{col} = volume of empty column = $V_0 + V_p + V_s$.

On the basis purely of geometrical structure SG60F is superior to Hypersil and the other materials for exclusion chromatography because of its unusually high ratio of $V_p/V_0 = 1.53$, the ratio for the other materials ranging from 0.70 to 1.00. Since the peak capacity, θ , for any separation by exclusion chromatography on a column of N plates²⁰ is given by

$$\theta = 1 + 0.6 \sqrt{N} \log_{10} \left(\frac{V_p + V_0}{V_0} \right) \quad (8)$$

TABLE III

GEOMETRICAL PROPERTIES OF POROUS SILICAS

V_0 = Extraparticle void volume; V_p = pore volume; V_s = volume of solid structure; V_{col} = empty column volume = $V_0 + V_p + V_s$.

Silica gel	Volumes relative to extra particle volume		Volumes as a fraction of tube volume			V_s $V_p + V_s$
	V_p/V_0	V_s/V_0	V_0/V_{col}	V_p/V_{col}	V_s/V_{col}	
Hypersil	0.81	0.52	0.43	0.35	0.22	0.39
SG60F	1.53	0.41	0.34	0.52	0.14	0.21
PSM Materials (average)*	0.70	0.70	0.42	0.29	0.29	0.50
Lichrosphere (average)*	0.96	0.55	0.40**	0.38	0.22	0.37
Porasil D***	1.00	0.50	0.40	0.40	0.25	0.38

* Data from ref. 21.

** Assumed.

*** Data from ref. 5.

it is calculated, for example, that SG60F will have 50% greater peak capacity than Hypersil when N is reasonably large. However, as shown by Table III, this arises partly because of the unusual low interparticle porosity of SG60F of only 0.34 compared to 0.40–0.43 for the other materials listed. This could be due to slight particle distortion and crushing during packing. This view is supported by our observations that SG60F collapsed if it was packed under a pressure in excess of 135 bar or used routinely at a pressure difference exceeding 75 bar.

Fragility is, of course, the expected consequence of a very low proportion of silica in the particle, 21% of the particle volume for SG60F compared to 37–50% for the other materials. It is thus evident that in designing a material for exclusion chromatography a careful balance must be struck between high porosity which implies fragility and high strength which reduces peak capacity.

(B) Determination of polydispersity

By eqn. 6 the polydispersity of a polymer standard can be obtained by measurement of h_{app} on columns of different lengths. Fig. 3 shows plots of h_{app} versus linear flow velocity for PS20K on Hypersil using columns of 55, 101 and 257 mm length. Fig. 4 shows corresponding curves for benzene. For benzene identical curves are obtained for the three columns of different lengths as expected when using a single pure substance. The curves for the polymer are different for each length, the longer the column the higher h_{app} , but significantly have the same gradients. Since the only velocity dependent part of h_{app} is h_{kin} this is as expected.

Fig. 5 shows h_{app} for various polymer standards plotted against column length at fixed eluent velocities. The points fall on good straight lines in agreement with eqn. 6.

Extrapolation to zero column length should provide values of h_{kin} . Such values are given in Table IV for the lowest eluent velocities used or those giving minimum values of h_{app} . The values are subject to fairly large errors due to extrapolation but

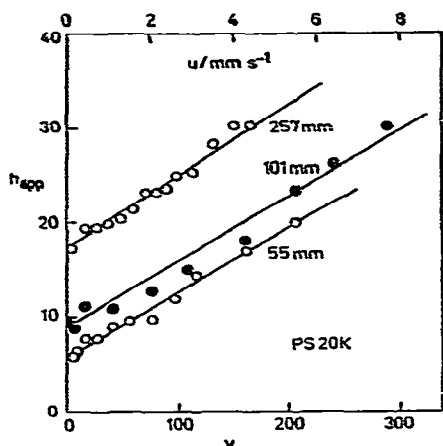


Fig. 3. Dependence of h_{app} upon v for PS20K eluted from Hypersil by dichloromethane from columns of different lengths as noted on lines.

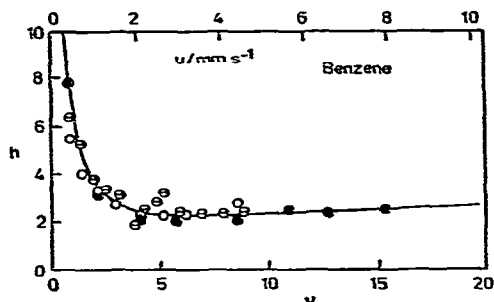


Fig. 4. Dependence of h upon v for benzene eluted from Hypersil by dichloromethane from columns of different lengths: \ominus , 55 mm; \bullet , 101 mm; \circ , 257 mm.

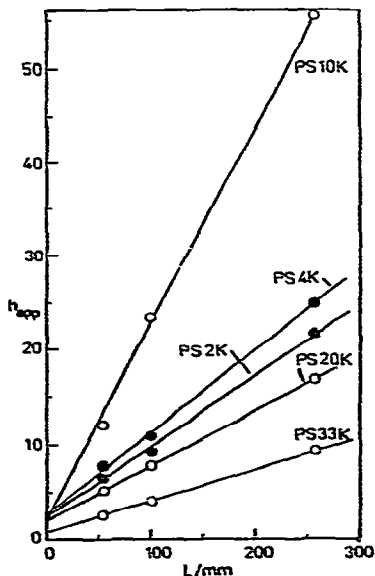


Fig. 5. Plots of h_{app} against column length, L , for different polymers: \circ , eluent velocity, extrapolated to zero; \bullet , eluent velocity 1.5 mm sec^{-1} . Packing, Hypersil; eluent, dichloromethane.

are consistent with the minimum value of h for benzene namely $h = 2.3$. Table IV lists the gradients of the plots of h_{app} versus L , the values of S/V_R calculated from the calibration graph and the derived values of the polydispersity P . These last values are seen to be very close to unity emphasising the excellence of the standards but at the same time confirming that under most circumstances the major contribution

TABLE IV
VALUES OF h_{kin} AND P FOR POLYMERS ELUTED FROM HYPERSIL

Polymer standard	h_{kin}^*	Slope of h_{app} vs. L plot**	S/V_R ***	P
Benzene	2.3	—	—	—
PS2K	2	74	0.114	1.031
PS4K	3	87	0.123	1.031
PS10K	2	204	0.141	1.052
PS20K	2	57	0.156	1.013
PS33K	1	33	0.167	1.007

* Lowest values obtained by extrapolation of h_{app} versus L plots to zero length.

** From Fig. 5. Units are m^{-1} .

*** From Fig. 2 ($S/V_R = d \ln V_R/d \ln M$).

to band dispersion in the exclusion chromatography of such materials arises from their polydispersity not from kinetic effects. The values of P derived by us are not in good agreement with those calculated from M_w and M_n given by the suppliers.

We suggest that measurement of h_{app} for a range of column lengths is the most accurate and reliable way of determining P for polymer standards, being far superior to independent measurement of M_w and M_n .

(C) Determination of the mass transfer coefficient, C

Fig. 3 shows a typical set of reduced plate height-velocity curves for a single polymer standard eluted from columns of different lengths. These curves are mutually parallel as predicted by eqns. 3 and 6. They should in fact be parallel to the curve for h_{kin} versus velocity, their displacement, as shown in section B, being determined by the polydispersity of the polymer standard. The polydispersity contribution to h_{app} may be determined for each column length from Fig. 5 or Table IV and can then be subtracted from each experimental value of h_{app} to isolate h_{kin} . Fig. 6 shows plots of h_{kin} versus v obtained in this way for all columns and solutes. The onset of the rise in h_{kin} at reduced velocities below 5 is noted for benzene, PS2K and PS4K. For the higher polystyrenes there is insufficient data at low values of v for the rise to be seen. For relatively high reduced velocities the main velocity dependent contribution to h_{app} comes from the mass transfer term of eqn. 3, C being identifiable with the gradient of each curve at high values of v . Evidently C is little dependent upon k'' . Table V lists the experimental values of the gradients of the curves. From the known values of k'' , values of D_m/D_s can be calculated using eqn. 3a. The values range from ca. 9 to 22 suggesting that even for the low-molecular-weight polymers diffusion appears to be severely restricted within the pores of the silica gel.

On theoretical grounds one might expect diffusion within a porous matrix to be somewhat restricted and indeed from the work of Knox and McLaren²² and Fluendy and Horne²³ a ratio D_m/D_s of ca. 1.5 might be expected. This value arises partly from the tortuosity of diffusion paths and partly from obstruction to diffusion by the alternation of narrow ports and wider chambers. Recently Van Krefeld and Van den Hoed¹⁸ measured D_m/D_s for partially excluded polymers by carrying out elution of polymers through beds of 60- μm Porasil using extremely high reduced velocities when Giddings' "near-equilibrium" assumption was far from true¹⁰. Under

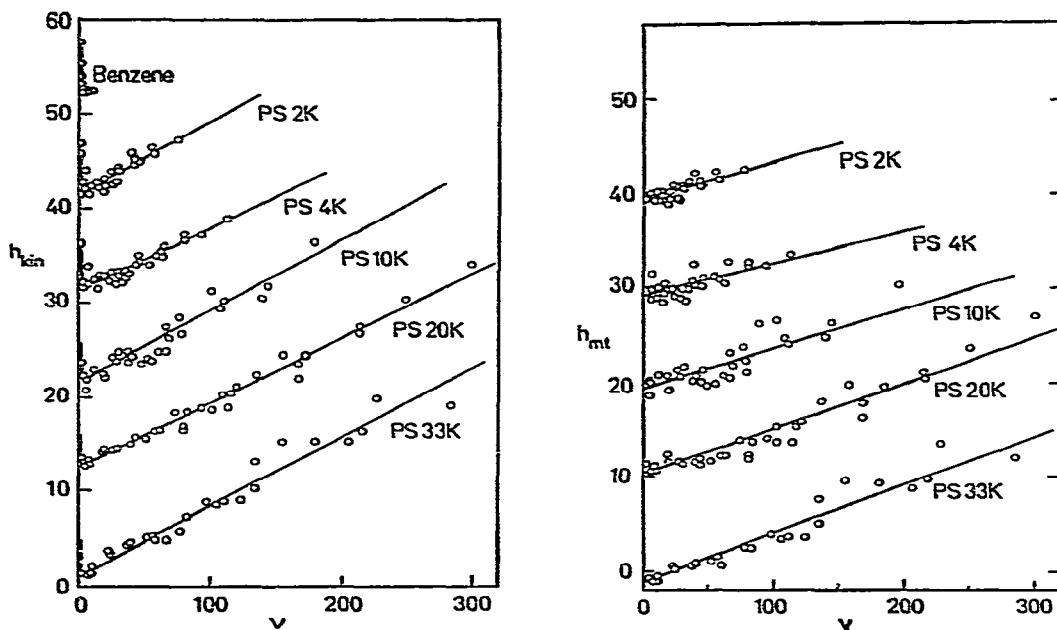


Fig. 6. Plots of h_{kin} against v for different polymers, $h_{kin} = h_{app} - h_{poly}$ where h_{poly} is calculated for each column length from Fig. 5. Data for all column lengths are included. Packing, Hypersil; eluent, dichloromethane.

Fig. 7. Plots of h_{mt} against v for different polymers $h_{mt} = h_{kin} - 2/v - v^{\frac{1}{2}}$. Data as for Fig. 6.

TABLE V
MASS TRANSFER COEFFICIENTS C ON HYPERSIL

Sample	k^{**}	K^*	$100 \times \text{gradient}$		D_w/D_s	
			Uncorrected**	Corrected***	Uncorrected [†]	Corrected [†]
Benzene	0.79	1.00	—	—	—	—
PS2K	0.58	0.74	9.3 ± 0.8	5.0 ± 0.8	10.7 ± 0.1	6.5 ± 1.1
PS4K	0.52	0.66	7.2 ± 0.6	4.5 ± 0.6	9.6 ± 0.8	6.0 ± 0.8
PS10K	0.32	0.41	7.2 ± 0.7	4.7 ± 0.7	11.8 ± 1.2	7.7 ± 1.2
PS20K	0.22	0.28	7.0 ± 0.25	5.3 ± 0.25	14.2 ± 0.7	10.7 ± 0.7
PS33K	0.12	0.15	7.1 ± 0.4	5.4 ± 0.4	22.2 ± 2.1	16.9 ± 1.8

* From calibration graph.

** Best fit gradients Fig. 6; limits are standard errors.

*** Best fit gradients Fig. 7; limits are standard errors.

[†] Errors include standard error in gradient and an estimated standard error of 0.01 in k^*

their conditions the peaks were severely tailed. However, for such peaks, where the tailing is due only to kinetic factors, as Kubin²⁴ showed, the first moment, μ_1 (the equivalent parameter to k^*) should still be independent of velocity. This was confirmed by Van Krefeld and Van den Hoed¹⁸. Kubin also showed that $L\mu_2/\mu_1^2$ is equivalent to the plate height, H (where μ_2 is the second moment) and is given by an equation essentially identical to 3. At the reduced velocities used by Van Krefeld and Van den Hoed (10^3 to 10^5 for polymers and 70–1300 for toluene) only the mass

transfer coefficient C could be determined and from this D_m/D_s could be found. Van Krefeld and Van den Hoed¹⁸ found that D_s had the form:

$$D_s = D_s^0 + D_1 u \quad (9)$$

The velocity dependent part of D_s was important at the highest velocities and D_1 was greatest for polymers of highest molecular weight. The values of D_1 were consistent with the hypothesis that the acceleration of mass transfer represented by $D_1 u$ arose from flow through the pores within the particles themselves. The higher values of D_1 for the larger polymer species are then explainable since the larger molecules are restricted to the largest pores through which the flow will be fastest. It is then the values of D_s^0 which have to be compared with our values of D_s obtained at intermediate reduced velocities (up to *ca.* 400).

Van Krefeld and Van den Hoed¹⁸ found $D_m/D_s^0 \approx 1.5$ for toluene as expected from the theory of obstructed diffusion in a porous medium while for the polymers D_m/D_s^0 ranged up to 9 for the most excluded material having $k'' = 0.11$.

Two explanations may be considered for the higher values obtained in this work.

(1) Because of the narrowness of the pores in Hypersil (*ca.* 120 Å) it is possible that the layer of eluent close to the internal surface of the material is highly structured and that diffusion within the layer is slow. This explanation was discounted as a result of experiments with SG60F which has much larger pores (*ca.* 600 Å) and so should show negligible effects due to the influence of surface on the structure of eluent in the pores. Following the same procedure as for Hypersil D_m/D_s was found to be *ca.* 15 for PS4K. A similar value may be calculated from the data of Kirkland and Antle²⁵ for PS2K eluted from a porous silica with a pore diameter of 400 Å.

(2) In calculating C_s it has been assumed that the other terms in eqn. 3 are negligible. A glance at Fig. 6 shows that such an assumption is likely to be true for the high-molecular-weight polymers where the majority of data correspond to high reduced velocities, but unlikely to be true for the lower MW polymers where the upper values of v are fairly low. In fact C should be calculated as:

$$h_{mt} = Cv = h_{2vp} - B/v - Av^2 \quad (10)$$

Typical values of A and B are 2 and 1 respectively¹³. Fig. 7 shows plots of h_{mt} according to eqn. 10 against v . The resulting "corrected values" of D_m/D_s are given in Table V. These values are now more self consistent although still consistently higher than those found by Van Krefeld and Van den Hoed¹⁸, as shown in Fig. 8. There seems no simple explanation of this difference unless it arises from differences in the geometrical structures of Hypersil used by us and Porasil used by Van Krefeld and Van den Hoed.

(D) Use of a monodisperse polymer

As a final check on the validity of the above analysis which separates dispersion due to kinetic effects from that due to polydispersity we attempted to prepare

monodisperse polymers by semi-preparative chromatography from the polymer standards.

Using PS3K an attempt was made to prepare a very narrow cut by means of exclusion chromatography. The plate height-velocity curve for this material is compared to that of the original standard in Fig. 9. Some improvement has been achieved but h is still well above that expected for a monodisperse polymer. Using the method described in section B the polydispersity of the original sample of PS3K is found to be 1.027 and that of the fractionated sample 1.014. Even if an infinitely narrow cut had been made one would have not achieved a monodisperse sample but rather one for which $h_{\text{poly}} = h_{\text{kin}}$ (see eqn. 6b). In the present instance the lowest possible value of polydispersity would then have been 1.005.

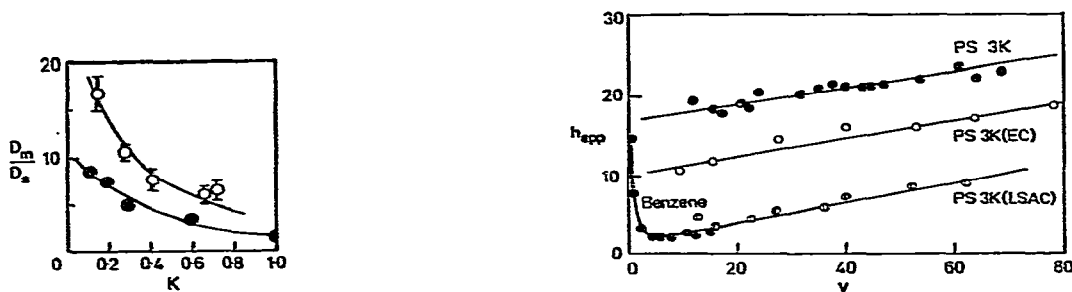


Fig. 8. Dependence of D_w/D_n upon degree of permeation, K . \circ , This work, standard vertical bars show \pm standard error; \bullet , data of Van Krefeld and Van den Hoed¹⁸.

Fig. 9. Plots of h_{app} versus v for PS3K fractions compared with h, v curve for benzene. Samples: PS3K original standard; PS3K (EC) standard fractionated by exclusion chromatography; PS3K (LSAC) standard fractionated by liquid-solid adsorption chromatography (see Fig 10). Packing, Hypersil; eluent, dichloromethane.

A more successful fractionation was achieved by adsorption chromatography. By using an eluent of pentane-dichloromethane (80:20) the adsorption chromatogram shown in Fig. 10 was obtained from which almost pure single oligomers could be isolated. The plate height-velocity curve for one such oligomer is shown in Fig. 9 where it is compared with the curve for benzene. It is clear that the two curves are very similar as predicted for solutes with similar values of k'' . Attempts to fractionate the PS10K standard by adsorption chromatography were less successful but the polydispersity was nevertheless reduced from *ca.* 1.063 to *ca.* 1.017.

(E) Dispersion of Excluded Polymers

While the dispersion of samples of partially excluded polymers is well explained by chromatographic theory developed for retained solutes, the dispersion of samples of excluded polymers cannot be so explained. The theory of chromatography predicts that with $k'' = 0$ the kinetic plate height should be given by

$$h_{\text{kin}} = B/v + Av^{\frac{1}{2}} \quad (11)$$

and that h_{poly} should be zero since (S/V_R) must be zero. Fig. 11 shows the behaviour of h_{app} as a function of velocity for the excluded polymer PS200K eluted from

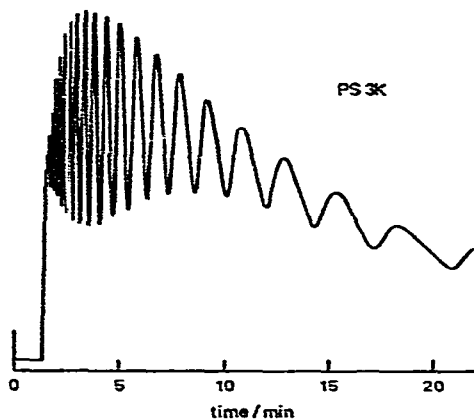


Fig. 10. Liquid-solid adsorption chromatogram of PS3K on Hypersil. Eluent, pentane-dichloromethane (80:20); column, 101 × 7 mm.

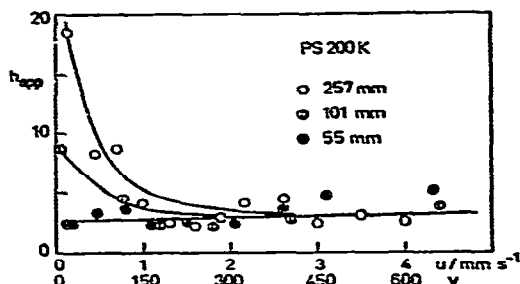


Fig. 11. Plot of h_{app} versus u and v for polymer PS200K excluded from Hypersil on columns of different lengths as shown. Packing, Hypersil; eluent, dichloromethane.

Hypersil. Similar behaviour was noted for two other excluded polymers PS400K and PS2700K. For all three polymers the following features was observed.

(1) For the shortest column (55 mm) h_{app} rose slowly with velocity and had a value of *ca.* 3 for PS200K and PS470K much as expected for a well behaved excluded solute obeying eqn. 11. The high velocity values for PS2700K were from 7 to 12.

(2) h_{app} at high velocities was more or less independent of column length.

(3) At low velocities h_{app} with longer columns (101, 257 mm) showed a rise as the velocity was reduced. This looks superficially like the rise expected from the *B* term of eqn. 3 or 11 but it occurred at far too high reduced velocities (200 to 1000 increasing with the MW of the polymer).

(4) The reduced velocity at which the minimum *h* occurred was higher the longer the column (see Fig. 11) and the higher the molecular weight.

(5) With the longer columns, as the elution velocity was reduced, peaks became tailed and generally misshapen.

We propose that these effects can be explained qualitatively by assuming that some very slow processes can occur to a proportion of the polymer molecules during their migration along the column, the time constant of this process being similar to the elution time of the sample.

These processes might be envisaged as the partial penetration of otherwise excluded polymer molecules into the outermost pores of the packing material. Since this would require partial unravelling of the polymer chain so that it could enter the pores whose diameter is much less than the hydrodynamic radius of the polymer the process is likely to be very slow and might have a time constant comparable with the time of passage of the sample through the column. Thus the longer the sample is in the column and the larger the molecule, the more likelihood there will be that a given molecule is trapped in this way. We can thus explain qualitatively at least why the effect is most noticeable in the longer columns, why it sets in at the lowest velocities only and why the peaks become increasingly tailed as the velocity is

reduced. The penetration will not be controlled by the diffusion rate of the polymer as a whole but by the rate at which discrete parts of the molecule (with a MW comparable to that of a just-excluded polymer) can rearrange themselves to penetrate the outermost pores. Since the larger the polymer molecule the greater its surface which can contact a particle of packing, the greater the chance that a segment of such a molecule can penetrate the outer pores of the packing. While this process is likely to be slow the additional volume which becomes accessible as a result of this process is nevertheless very small so that there is no change in elution time although peak tailing occurs due to the slow desorption of the partially trapped molecules.

(F) Column overloading

In retentive chromatography overloading of the stationary and mobile phase results in an increase of the apparent plate height. As shown by Done²⁶ this becomes noticeable in high performance columns when the load of solute is of the order of 1 μg of solute per gram of an adsorbent such as silica gel. Fig. 12 shows that similar effects are observed in exclusion chromatography. Once again the apparent plate height rises linearly with increase in load. In contrast to overloading in adsorption chromatography, which becomes worse as k' increases, overloading in exclusion chromatography becomes worse as k'' decreases and molecular weight increases. The load for a significant increase in h_{app} is again of the order of 1 μg per cm^3 of column packing.

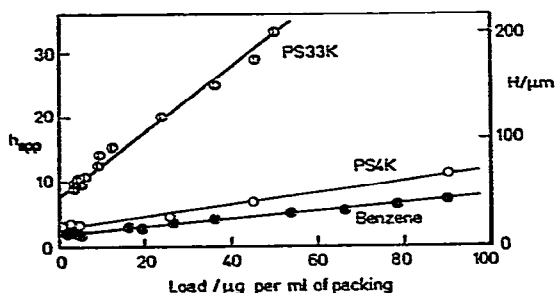


Fig. 12. Effect of sample load on h_{app} for elution of polymer standards and benzene. Packing, SG60F; eluent, dichloromethane; column, 125 \times 7 mm; eluent velocity, 1.5 mm s^{-1} .

CONCLUSIONS

From sections A–D it is concluded that the kinetic equations derived for retentive chromatography are completely applicable to exclusion chromatography and can be used to predict precisely the value of the plate height arising from such processes. In using eqn. 3 one should, however, allow for the fact that diffusion of polymers within networks from which they are substantially excluded may be up to twenty times slower than in bulk-eluent.

We also conclude that the polydispersity of polymer standards is readily obtained by plotting the apparent plate height against the column length or more simply by subtracting a calculated kinetic contribution from h_{app} and then using eqn. 6.

The dispersion of bands of excluded polymers is not readily explained, but at low velocities appears to be connected with slow processes occurring to solute molecules with time constants comparable to the elution time from the column.

Overloading in exclusion chromatography occurs at much the same level of sample size as in retentive chromatography but becomes more serious the higher the molecular weight of the polymer.

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