should increase with increasing molecular mass.

The shape of the VITX-120120 particles from the column and of fresh CPG material was investigated by scanning electron microscopy. As expected, the VITX material shows the smaller pore diameter. Single pores having a significant greater diameter, which could explain the small values of $H_{\text{perm}}^{\text{flow}}$, were not found. The particle form and the size distribution of both materials differ considerably, although they presumably have the same producer. While the CPG material, which probably had been sieved in a nonporous state, has the shape of broken pieces of glass and a relatively narrow size distribution, the edges of the VITX material were rounded and a large fraction consisted of pieces having a diameter of but a few micrometers. This damage of the material might be the result of the sieving of the porous material by the manufacturer or of a wear-up of the packing during prolonged use.

The VITX-120120 column had shown a considerably higher pressure gradient than the other columns. Presumably the low permeation dispersion of the VITX-120120 material is the result of partly clogged interstitial pores, which leads to an increase of u_s.

The experiments thus confirm the significance of convective transport in the internal pores at packing materials having high exclusion limits and working with high linear flow rates. The results, however, show an improvement of the broadening behavior of samples by convective transport, which is much higher than would be expected from simple models. The reason for this gratifying discrepancy might be the particular shape of the particles and the pores of the materials studied.

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Mass Transfer of Macromolecules in Steric Exclusion Chromatography. 3. Influence of the Interstitial Volume on Retention

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ABSTRACT: As particle diameter of size exclusion chromatography (SEC) packings is decreased, elution behavior of high molecular mass samples can be influenced by interstitial effects. The retention of macromolecules at different columns is shown to deviate in the region of high molecular mass from the behavior predicted by classical SEC theory. Three additional effects are shown to be operating: external exclusion, deformation, and degradation of macromolecules in the interstitial volume.

1. Introduction

In steric exclusion chromatography, molecules which are totally excluded from the internal pore volume are eluted at the interstitial volume, V_0. The distribution of permeating molecules between the internal and the interstitial volumes is governed by the thermodynamic coefficient K of the equilibrium partition between the internal pore and free solution. Thus in SEC, the elution volume is given by

$$V_e = V_0 + KV_i \qquad \text{where } 0 \leq K \leq 1 \tag{1}$$

The elution volume is independent of the flow rate if no hydrodynamic fractionation, as described in a preceding article of this series,[1] takes place.

With the development of small-particle packings for HPLC, the diameter of the interstitial pores decreases and the flow gradients which act on the sample molecules increase. As will be shown here, in the modern SEC of macromolecules having a high molecular mass, processes occurring in the interstitial volume might influence retention.

One effect that influences the elution volume of macromolecules has to be eliminated before mechanistic studies in the high molecular mass region can be made: the concentration effect, which is the result of nonlinear distribution isotherms and viscous fingering in the interstitial volume. Thus, in preliminary studies a concentration for each sample was determined at which no change of the elution curve was found compared with a sample having twice the concentration.

In the first part of this paper, external exclusion will be discussed as an effect of the interstitial pores which is not flow rate dependent. In the second part, the flow rate dependent effects found at other packings will be dealt with.

2. External Exclusion

2.1. Theory. In modern SEC, packings having a particle diameter down to 5 μm are used. The diameter of the internal pores can be as large as 0.4 μm. Macromolecules which are markedly excluded from pores having such a large diameter should also partly be excluded from

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Table I
PS Standards Used at the TSK G6000H6 Column with Molecular Masses According to the Manufacturer

manufacturer[a]	$\bar{M}_n \times 10^{-3}$	$\bar{M}_w \times 10^{-3}$	$\bar{M}_w/\bar{M}_n$	V_e, mL
W	3.1	4.0		12.49
W	9.6	10.0		11.34
W	20.0	20.8		10.58
W	36	33		9.71
W	111	111		9.59
W	193	200		9.54
W	773	867		9.50
T	1260		1.05	9.34
T	2880		1.09	9.21
T	5050		1.02	9.17
T	7500		1.02	9.04
T	9500		1.03	8.97
T	16100		1.16	9.67

[a] W = Waters Associates; T = Toyo Soda.

the outer zones of the interstitial pores; i.e., the concentration in that region is less than in the center of the pores. Therefore, the sample molecules are in the mean in regions of higher local flow rate than smaller molecules and are eluted earlier. If the molecules are completely excluded from the internal pores, their elution volume can thus be smaller than V_0.

DiMarzio and Guttman[2] proposed the use of the exclusion of molecules from the walls of capillaries for a new type of separation, the "hydrodynamic fractionation". Actually, Mori, Porter, and Johnson[3] demonstrated the separation effect with polystyrene samples at bundles of glass fibers and Small[4] with polymer lattices at spherical packing materials which the samples could not permeate.

The drawback of the pure hydrodynamic chromatography in the interstitial volume of packing lies in the very narrow and inflexible separation range. A significant improvement could be obtained by a combination of external exclusion and exclusion in the internal pores of the packing, as has been described theoretically by Guttman and DiMarzio.[5]

External exclusion can also lead to errors of the values of the interstitial volume V_0. The interstitial volume is usually determined with samples which are totally excluded from the internal pores. In the case of external exclusion the elution volume found does not equal V_0.

The action of steric exclusion in SEC can be proved by the determination of a molecular mass/elution volume calibration curve at materials having small internal pores and with samples of high molecular mass. In the case of external exclusion, the elution volume should decrease with increasing molecular mass even if the samples are totally excluded from the internal pores.

2.2. Experiments and Discussion. The packing material which was investigated for external exclusion was the polystyrene gel TSK G3000H6 (Toyo Soda), whose spherical particles have a diameter of about 10 μm.[6] The material was received ready packed in a column having a $^3/_8$-in. i.d. and a 2-ft length.

As a solvent THF was used for the samples toluene and anionically polymerized polystyrene from Waters Associates and Toyo Soda (Table I). For toluene and the PS samples with $\bar{M} < 10^6$ a concentration of 600 ppm was used, for the samples with $10^6 < \bar{M} < 2 \times 10^6$ a concentration of 125 ppm was used, and for the samples with $\bar{M} > 2 \times 10^6$ a concentration of 31 ppm was used. The injection volume was 50 μL.

The elution volume of toluene was determined at a nominal flow rate of 1.0 mL/min. The measurement of the real flow rate was carried out with a volumetric flask. Toluene was added to the PS samples as an internal standard for the determination of the flow rate and their elution volume.

The elution volumes found (measured at the peak maximum) are included in Table I. The "exclusion volume" (the interstitial volume V_0) at the column investigated seems to be reached at a molecular mass of about 200 000. Until the molecular mass of 10^7 a further decrease of the elution volume takes place. The sample having $\bar{M}_n = 1.61 \times 10^7$ showed a higher elution volume together with a greater broadening and a concentration dependence of the form of the elution curve even at the low concentration used. The elution behavior of this sample of extremely high molecular mass will not be discussed here.

In the case of the column investigated, external exclusion leads to a decrease of the elution volume of 0.57 mL in the range of molecular mass from 5×10^5 to 10^7. The internal pore volume can be approximated by using the elution volume of the toluene sample; it is about 8.80 mL. External exclusion thus only makes up but a small part of the separation range. It has to be considered, however, that external exclusion is a gratis adjunct (small particle diameters are used to minimize the peak broadening) and that the effect adds at the use of a column set. With the whole set the contribution of the external exclusion to the separation, particularly in the region of high molecular masses, where peak broadening is high due to the small diffusion coefficient, can well play a substantial role.

A reduction of the radius of the packing material for an enlargement of the external exclusion can lead to difficulties due to the resulting high-pressure gradient. Besides, a too small diameter of the internal pores or of the frits at the endings of the columns might lead to a delay of the sample molecules or to clogging of the column. The increase of the elution volume of the sample with the highest molecular mass might well be the result of such effects.

The interstitial volume V_0 of packings having a small particle diameter can only be calculated directly from excluded samples with relatively low molecular mass, and if the internal pores are large, external exclusion has to be taken into account. In a former article of this series,[6] the exclusion volume at the TSK GH6 columns having larger pore diameters was therefore not taken directly from the elution volume of an excluded sample, but this volume was corrected for external exclusion by subtracting the difference found at column G3000H6 between this sample and a sample with $\bar{M}_w = 2 \times 10^5$. The latter sample was just excluded from column G3000H6 and presumably showed but a neglegible external exclusion.

3. Deformation and Degradation

3.1. Theory. If the gradient of the flow rate in the interstitial volume and the size of the macromolecules are sufficiently large, a deformation of coiled macromolecules might result. By the deformation the entropy of the molecules in the interstitial volume decreases and with it the difference of entropies between the interstitial and the internal pore volume. Thus the distribution coefficient and the elution volume both increase. This effect has been postulated by Metzner[7] for the interpretation of results obtained for studies on polymer flooding and has not yet been identified in SEC. It should manifest itself as an increase of the elution volume with increasing flow rate.

If the deformation of the macromolecules is too great, a degradation of the macromolecules takes place. This effect was proved experimentally by Slagowski, Fetters, and McIntyre[8] and Kirkland.[9] The procedure Kirkland used for the proof of the degradation seems especially

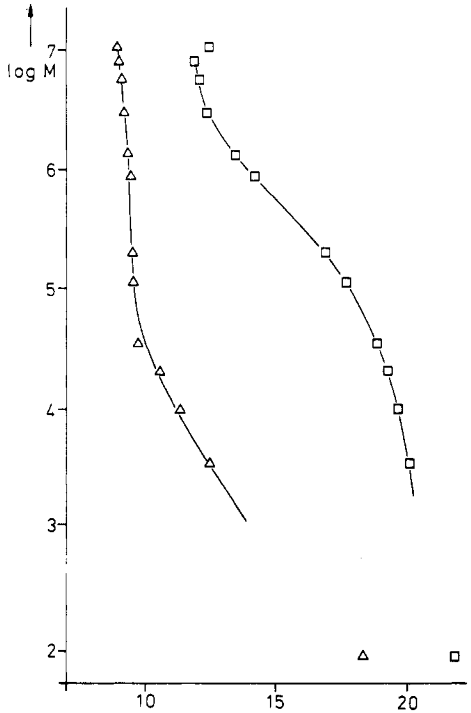

Figure 1. Calibration curve of the TSK columns G3000H and G6000H; flow rate 1.0 mL/min.

simple and reliable: he injected polymer samples at two different flow rates. At the minor flow rate he found a smaller elution volume, which might be the result of degradation. The sample eluted with the high flow rate was reinjected at the minor flow rate. The elution volume found was more than that of the fresh sample injected at the slow flow rate; i.e., at the higher flow rate a degradation has taken place.

3.2. Experiments and Discussion. The behavior of high molecular mass samples was investigated at two columns having a large internal pore diameter. The columns were 2-ft long × $^3/_8$-in. i.d. filled with the polystyrene gel TSK G6000H6 (Toyo Soda) and 50-cm long × 2.6-mm i.d. filled with VITX-120120 (Perkin-Elmer), a 36–44-μm sieving fraction of pieces of broken glass.

In the case of the VITX column, $CHCl_3$ was used as a solvent; in the case of the TSK column, THF was the solvent.

The calibration curve of the columns was established at a nominal flow rate of 1.0 mL/min, using anionically polymerized polystyrene standards (Figures 1 and 2). For both columns the slope of the curve increased from the molecular mass of about 10^6 on until at last the curve was vertical; in the case of the TSK column in the range $M > 8 \times 10^6$ the elution volume even increased.

The "exclusion volume" obtained from these measurements was 12.08 mL at the TSK column and about 2.2 mL at the VITX column. As on columns with smaller pore exclusion volumes of about 9.5 mL, about 1.2 mL was found; in the case of both columns investigated the increase of the calibration curve does not seem to be the result of real complete exclusion.

The elution volume of some samples was investigated at different flow rates. The exact flow rate and the elution volume were determined with toluene.[6]

In the case of the TSK column 50 μL of a 31-ppm solution of a PS standard from Toyo Soda having, according to the manufacturer, $\bar{M}_w = 5.05 \times 10^6$ and $\bar{M}_w/\bar{M}_n = 1.02$, was injected. At the VITX column 25-μL aliquots each of a 125-ppm solution of a PS standard from Waters Associates with $\bar{M}_w = 2.61 \times 10^6$ and $\bar{M}_n = 1.99 \times 10^6$ and a 63-ppm solution of a PS standard from Toyo Soda with $\bar{M}_w = 9.50 \times 10^6$ and $\bar{M}_w/\bar{M}_n = 1.03$ (data according to the manufacturer) were injected.

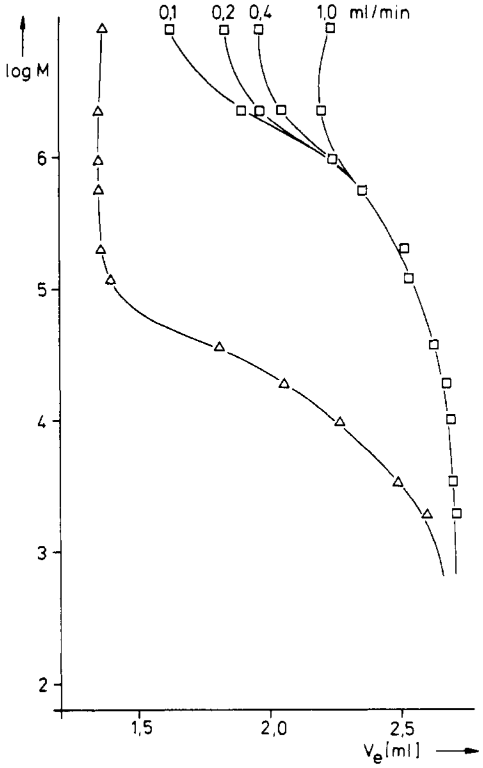

Figure 2. Calibration curve of the VITX columns VITX-1068 and VITX-120120; flow rate 0.2 mL/min except for the two highest molecular mass standards at VITX-120120 (the other standards showed no significant flow rate dependence).

Table II
Change of the Elution Volume with Flow Rate[a]

column standard	flow rate, mL/min	first injection V_e, mL	reinjection V_e, mL
TSK G6000H6	0.2	10.7	10.6
$\bar{M}_w = 5.05 \times 10^6$	0.5	11.7	
	1.0	12.1	
VITX-120120	0.1	1.90	
$\bar{M}_w = 2.61 \times 10^6$	0.2	1.97	2.14
	0.4	2.05	
	1.0	2.20	
VITX-120120	0.1	1.62	
$\bar{M}_w = 9.50 \times 10^6$	0.2	1.84	2.12
	0.4	1.96	
	1.0	2.24	

[a] The reinjected samples were first injected at 1.0 mL/min.

A 500-μL quantity of these standards was injected at a nominal flow rate of 1.0 mL/min and collected, and 50 and 25 μL, respectively, were reinjected at a flow rate of 0.2 mL/min.

The results of these measurements are given in Table II. Both columns show an increase of the elution volume with increasing flow rate.

In the case of the TSK column the molecular mass does not change at the higher flow rates, as the elution volumes of the reinjected samples show. The increase could be the result of hydrodynamic fractionation or of deformation of the molecules. An increase of the elution volume with flow rate could be obtained if the entering of the molecules into the internal pores is determined by flow.[1] In that case, however, the transport through the pores would be determined by flow even for lower molecular mass samples. The measurement of the permeation dispersion at that column, however, did not indicate convective transport for these samples.[6]

Because of the still approximate character of the theoretical calculations for hydrodynamic chromatography, the possibility of the action of hydrodynamic fractionation of the high molecular mass samples studied cannot be totally ruled out, but it seems more probable that the effect found at that column is based on the deformation of the macromolecules in the interstitial volume.

In the case of the VITX column the samples reinjected at the slower flow rate showed a higher elution volume than the freshly injected ones. That means that at the higher flow rate a degradation of the samples had taken place on the column. The fact that the injection volume of the reinjected sample is still less than the elution volume of a fresh injected sample at the high flow rate points to an additional effect, probably based on the reversible deformation of the macromolecules.

The distinct behavior of the samples at both materials investigated might be based on the fact that the macromolecules are subject to higher local shearing and stretching stresses at the edged VITX particles and are therefore more easily degraded than at the spherical TSK particles.

The effects found should show up especially in the case of high-speed SEC and for samples of extremely high molecular mass. Here there are only but a few investigations published. While several investigators found an increase of the elution volume with increasing flow rate,[8–11] Kato and co-workers[12] could separate PS samples of molecular masses ranging from 10^6 to 10^7 at a flow rate of 0.5 mL/min at the material TSK G6000H8 (particle diameter about 8 μm) without any problems. The reason for this might be the solvent used by the authors, methyl ethyl ketone. MEK is nearly a Θ solvent for PS, while $CHCl_3$ and THF are good solvents. A decrease of the coil dimensions brings about a decrease of the forces acting at the molecule and an increase of the stability of the coil.

SEC materials can thus fail in the region of high molecular mass in spite of sufficiently large internal pores. Working in this region, one has to consider the possibility that deformation or degradation effects demand a decrease of the flow rate or a change of the solvent, respectively, on the column.

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Rates of Intramolecular Collision between Terminal Groups of α,ω-Dianthrylpolystyrene in Benzene and Cyclohexane Solutions As Studied by Triplet–Triplet Absorption Measurements

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ABSTRACT: Rate constants of intramolecular end-to-end collisions, k_{intra}, in α,ω-dianthrylpolystyrene have been measured in benzene and cyclohexane solutions by following the rate of decay of triplet-excited anthryl groups. Polymer solutions were irradiated with 25-ns flashes of 347-nm light produced by a ruby laser. The decay of the triplet–triplet absorption after the flash was recorded as a function of time. In order to minimize intermolecular interactions, very dilute polymer solutions (1×10^{-5}–3×10^{-6} mol/L) were used. The values of k_{intra} were in the range 2×10^5–1×10^3 s^{-1}. In the case of benzene solutions at 22 °C, k_{intra} decreased with increasing degree of polymerization, N, according to $k_{intra} = (\text{const})N^{-n}$, with $n = 1.0 \pm 0.06$ for N covering the range 110–3000. In the case of cyclohexane solutions at 34 °C (Θ condition), a more complicated function holds for the decrease of k_{intra} with increasing N. The dependence can be approximately described by $k_{intra} = (\text{const})N^{-n}$, with $n = 1.5$ for $N \leq 300$ and with $n = 1.0$ for $N \geq 300$. A theoretical treatment taking into account the short-time fluctuation of chain segments is presented which explains the dependence of k_{intra} on N in the present case as well as in other cases reported previously in the literature.

I. Introduction

Time-resolved luminescence and absorption spectroscopy is a powerful tool for the study of structure, mobility, and kinetics of reactions of both synthetic and biological macromolecules.[1] If intramolecular reactions between the terminal groups of a polymer molecule occur as diffusion-controlled reactions, the rate constant of these reactions may be considered as a measure of the conformational mobility of the polymer chains. Photophysical processes[2–6] as well as electron-transfer processes in connection with ESR measurements[7] have proved suitable as probes for the study of intramolecular end-to-end collisions

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