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Mass Transfer of Macromolecules in Steric Exclusion Chromatography. 2. Convective Transport in Internal Pores (Hydrodynamic Chromatography)

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ABSTRACT: The transport of solute molecules through internal pores in steric exclusion chromatography is, under certain circumstances, not caused by diffusion but by convection ("hydrodynamic chromatography"). This results in a change of the separation mechanism and leads to an acceleration of the mass transfer. The retention behavior of macromolecules in hydrodynamic chromatography is examined. A drastic decrease of the peak broadening compared with diffusional transport in the pores is postulated. This effect is demonstrated to be operating at two porous glass packings having small particle diameters and large, regular pores.

1. Introduction

Under ordinary experimental conditions, the separation effect in steric exclusion chromatography (SEC) is based upon an equilibrium distribution between the interstitial volume and the stationary internal pore volume.[1,2] DiMarzio and Guttman[3-5] postulated an alternative separation mechanism based on the different residence times of molecules of distinct size in pores which are convectively permeated by the solvent. Verhoff and Sylvester[6] combined this idea with sieving effects at the pore entrance. As DiMarzio and Guttman have shown, this separation mechanism leads not only to different retention times but also to a decrease in peak broadening compared to the equilibrium distribution model.

As we show in this work, "hydrodynamic fractionation" is indeed operating in some specific SEC systems. First we give a critical evaluation of the theoretical concepts of this technique.

2. Theory

2.1. Mean Residence Time. Both DiMarzio and Guttman and Verhoff and Sylvester start with rather general assumptions which lead to complex calculations and, as Casassa[1] showed, different results. In this work, we start with the assumption that the flow rate in the internal pores of the separation material is small compared with the flow rate in the interstitial pores. The results of simple deductions based on this simplification will be compared with the results of the above-mentioned authors.

The chromatographic packing has large pores in its interstitial volume and small internal pores. If the internal pores are not large compared with the solute molecules, these may be sieved out from the solvent which flows into the internal pores. The sieving probability P shall be defined as the normed probability that a solute molecule whose center is situated at a stream line leading through an internal pore is actually entering the pore.

Solute molecules which have entered an internal pore flow through it with a mean linear velocity u_s', which, due to their higher probability of residence in regions of higher flow rate, is different from the mean velocity of the solvent in the pore, u_s.

The mean residence time t_e of a solute molecule is the sum of the time it travels in the interstitial volume, t_0, and the time t_i' it travels in the internal pores; t_i' is the fraction of the time t_i that a solvent molecule resides in internal pores.

A molecule which flows through internal pores has a shorter way to travel in the interstitial pores compared with an excluded molecule. If, however, the velocity in the interstitial pores is large compared with the velocity in the internal pores, this difference can be neglected.

Thus the following equation, which is independent of any particular model of pores, can be written:

$$t_e = t_0 + P(u_s/u_s')t_i \quad (1)$$

Again using the above-mentioned approximation, one gets from eq 1 by multiplying with the volumetric flow rate

$$V_e = V_0 + P(u_s/u_s')V_i \quad (2)$$

where V_e is the mean elution volume, V_0 the interstitial volume, and V_i the internal pore volume.

Detailed theoretical descriptions of the process of hydrodynamic fractionation are at this time not possible for two reasons: First, the structure of real porous networks

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is too complicated to be fully described by a theoretical model, and second, even for simple pore models the flow of molecules into and in the pores can only be described exactly for the case that the molecular diameter is relatively small compared with the pore diameter. Nevertheless, we will try to describe the separation process based on a simple pore model and on formulas given in the literature for small values of the ratio of the molecular radius, a, and the pore diameter, r. For greater values of a/r, plausible assumptions will be made.

If the solute molecules are carried into the internal pore so fast that diffusion processes play no role at the entrance, no sieving takes place for the molecules whose radius is smaller than the pore radius; i.e., $P = 1$.

Guttman and DiMarzio[4] have presented calculations for this case. Inserting the relation given by them for u_s/u_s' for the case of cylindrical pores into eq 2, one gets their result for the above-mentioned assumption that the flow rate in the interstitial pores is large compared with the flow rate in the internal pores:

$$V_e = V_0 + [2 - (1 - a/r)^2 - 2\gamma(a/r)^2]^{-1}V_i \qquad (3)$$

γ lies between 0 and 0.4, depending on the molecular shape.

Rigid spherical molecules will be excluded from the pore if their radius is larger than the radius of the pores; they will elute at the volume V_0. Flexible macromolecules might be dragged into the pores even if their effective coil radius is larger than the entry.

Verhoff and Sylvester[6] in their treatment of hydrodynamic fractionation stressed the sieving action at the pore entrance. This effect, which is based on a local equilibration process between molecules in free solution and in the pore, can be operating even if the travel of the molecules through longer distances, through the pores, is caused by flow and not by diffusion. As Casassa pointed out, Verhoff and Sylvester did not use the theory of ultrafiltration[7] adequately. If one takes their sieving constant for the case of cylindrical capillaries, $P = (1 - a/r)^2$, and inserts it into eq 2 together with the equation for u_s/u_s' given by Guttman and DiMarzio, one gets

$$V_e = V_0 + (1 - a/r)^2[2 - (1 - a/r)^2 - 2\gamma(a/r)^2]^{-1}V_i \qquad (4)$$

The sieving at the pore entrance due to Brownian motion of the solute molecules will be found at smaller values of u_s. If u_s becomes even slower, transport through the pores will also be carried out by diffusion. For the case of the equilibrium distribution of spherical molecules between free solution and cylindrical pores, the separating action of the u_s/u_s' term vanishes and one gets from eq 2 the formula for the case of equilibrium distribution:

$$V_e = V_0 + (1 - a/r)^2V_i \qquad (5)$$

However, only in this model does the sieving constant P equal the coefficient of equilibrium distribution; while P is determined by the entrance of the pore, the equilibrium coefficient is determined by the shape of the whole pore.

Equations 3–5 can be described by the general equation of steric exclusion chromatography

$$V_e = V_0 + KV_i \qquad \text{where } 0 \le K \le 1 \qquad (6)$$

if the rather improbable possibility is excluded that for bigger values of a/r the solute molecules are slowed down in the pores by frictional effects to an extent that is not compensated by a decrease of P so that K becomes greater than 1.

Normally one assumes that the flow rate in the internal pores is so slow that diffusional transport prevails and the separation is based on equilibrium-determined steric exclusion.

Hydrodynamic fractionation should be looked for in regions where the diffusion coefficient is sufficiently low and where the internal pore diameter is no longer small compared to the diameter of the external pores, i.e., the case of separation of macromolecules of high molecular mass.

The transition from diffusional to convective transport in and into the pores, respectively, by changing the flow rate should show up in a change of K. Until now, however, there existed no theory which allowed the calculation of the distribution coefficients in real pores; besides, other effects might result in a change of K with the flow rate, just as in the case of molecules of high molecular mass.

There is, however, another effect which depends on the mechanism of mass transfer in the internal pores: the broadening of the elution curve. As will be shown, this effect is more important for the effective separation of macromolecules than the influence of the separation mechanism on the elution volume itself.

2.2. Permeation Dispersion. In the case of diffusional transport, the plate height of the permeation dispersion (i.e., the broadening by transport processes in the internal pores) is proportional to the linear flow velocity in the interstitial volume, u:[9]

$$H_{perm}^{diff} = q(V_0KV_i/V_e^2)d_p^2u/D_s \qquad (7)$$

q is a form factor ($q = {}^1/_{30}$ for spherical materials), d_p is the particle diameter, and D_s is the diffusion coefficient in the stationary phase. Guttman and DiMarzio[5] have calculated the permeation dispersion for their capillary model, extending the mass balance calculation of Hermans.[10] Here, a simpler approach will be used which leads to similar results for the case of spherical particles. For the calculations, the random walk model of Giddings[11] will be used.

As shown by Giddings, H_{perm} is proportional to u and the mean time t_{ex} which elapses for a solute molecule entering the internal pores before it is back in the interstitial volume again:

$$H_{perm} = 2(V_0KV_i/V_e^2)\omega_\beta^2ut_{ex} \qquad (8)$$

The factor ω_β gives the ratio $\Delta u/u$, where Δu is the change of velocity during a "jump" of the molecule from the stationary into the mobile phase. As long as u_s is small compared with u, $\omega_\beta \approx 1$.

If one expresses the mean length of a pore through a particle as a function of d_p, using the constant ω_λ, one gets for the case of convective transport in the internal pores

$$t_{ex} = \omega_\lambda(d_p/u_s) \qquad (9)$$

From eq 8 and 9, one gets for H_{perm}^{flow}

$$H_{perm}^{flow} = 2\omega_\beta^2\omega_\lambda(V_0KV_i/V_e^2)(u/u_s')d_p \qquad (10)$$

If the sieving effect at the pore entrance can be neglected, $u_s' = u_s/K$ (compare eq 2). If K is independent of u, the ratio u/u_s' is it, too. Equation 10 thus corresponds to the "eddy dispersion" in the mobile phase. Here, too, the broadening is independent of the flow rate if the solute molecule is transported by flow and not by diffusion.[11]

In the case of spherical particles, ω_λ is the mean length of parallel chords through a sphere of diameter 1, ${}^2/_3$. With $\omega_\beta = 1$ one gets from eq 10

$$H_{perm}^{flow} = {}^4/_3(V_0KV_i/V_e^2)(u/u_s')d_p \qquad (11)$$

The changeover from diffusional to convective transport

in the internal pores can be described analogously to the corresponding changeover in the interstitial volume by the coupling equation given by Giddings:[11]

$$H_{perm} = (1/H_{perm}^{flow} + 1/H_{perm}^{diff})^{-1} \quad (12)$$

If one neglects the dependence of K on the separation mechanism, one gets by inserting eq 7 (with $q = {}^1/_{30}$) and eq 11

$$H_{perm} = (V_0 K V_i / V_e^2)\left(30\frac{D_s}{d_p^2 u} + \frac{3}{4}\frac{u_s'}{d_p u}\right)^{-1} \quad (13)$$

Convective and diffusional processes have about the same share on the transport of the solute molecule in the particle if

$$d_p u_s'/D_s = 40 \quad (14)$$

Below the region of changeover the permeation dispersion is proportional to the flow rate; above, it is independent.

A molecule that enters an internal pore of the packing possesses a distribution of residence time probabilities at the exit of the pore. This distribution brings about an additive broadening effect, which again can be calculated by eq 8, estimating t_{ex} for each exchange process, as is done by Giddings for the exchange processes in the mobile phase. The transfer into different regions of the internal pores (e.g., from the wall of the pore to the middle) might be effected by diffusion, while the transfer through the particle is predominantly effected by flow. At higher values of u_s, most of these transfer processes will be flow determined. As the part of the broadening which remains proportional to the flow rate there finally remains the broadening by diffusion into dead-end pores and that due to adsorption.

The equation of permeation dispersion calculated by DiMarzio and Guttman corresponds to eq 7 for the case of diffusional transport through the particle. For a certain range of velocities their relation equals eq 11 for flow transport (possessing, however, another form factor). For the region of changeover from diffusional to flow transport, these authors get an equation different from eq 12; this can be attributed to the fact that eq 12 is but an approximation for the real coupling process.

At higher flow rates the permeation dispersion, according to the equation of DiMarzio and Guttman, decreases again. This astonishing result is due to the fact that the authors contract the diffusion and the broadening due to Taylor diffusion to an "effective coefficient of diffusion". This procedure is not allowed in this case, as the Taylor diffusion does not determine the mean residence time in a particle but gives an additive broadening.

Hydrodynamic fractionation thus can be proven experimentally by the fact that the permeation dispersion is considerably smaller and less dependent on the flow rate than in the case of diffusional transport in the internal pores. The practical importance of the hydrodynamic fractionation lies in this reduction of the broadening compared to the normal process of exclusion chromatography, especially in the case of dispersions and samples with a very high molecular mass.

In the experimental section, we examine whether the separation mechanism of hydrodynamic fractionation is actually operating at separation materials for polymers of high molecular mass.

3. Experimental Procedures

The dependence of peak broadening on molecular mass of the sample and flow rate in the range 0.1–1.0 mL/min was examined by using the materials VITX-120120 and CPG-3000. Both materials are broken pieces of porous glass having a narrow pore size distribution.

The VITX material was received ready packed in columns from Perkin-Elmer; the CPG-3000 material (Electro-Nucleonics) was dry packed into a column having the same size as the VITX column (50-cm length and 2.7-mm i.d.).

The VITX material was a sieving fraction of 36–44-μm particle diameter; the pore diameter that Telepchak[12] determined for this material by Hg porosimetry was 1933 Å. The CPG material had, according to the manufacturer, a particle diameter of 36–75 μm and a pore diameter, determined by Hg porosimetry, of 2941 Å.

As solvents, $CHCl_3$ was used for the VITX column and THF for the CPG column.

The experimental procedure was the same as that described in the preceding article of this series.[13]

Table I
Regression Lines of the μ_2–$\dot{V}$ Relation

column	standard	offset, mL² × 10³	slope, mL min × 10³
VITX	2	7.89	5.16
	3	8.19	7.24
	4	8.81	5.20
	5	7.93	7.59
	6	9.15	5.59
	7	9.25	7.35
	8	12.22	4.16
	9	11.95	3.25
CPG	2	10.4	13.5
	4	12.0	22.7
	6	18.0	31.4
	7	22.5	31.7
	8	36.3	31.2
	9	37.5	35.6

4. Results and Discussion

For the samples having a molecular mass less than 10^6 no significant change of elution volume (measured at the peak maximum) with flow rate was found. Samples having a higher molecular mass, however, showed, especially in the case of the VITX column, a distinct increase of elution volume with increasing flow rate. This effect is the result of a degradation of the macromolecules, as will be shown in the last article of this series.[8]

The log M–K calibration curve in the range of $M < 10^6$ showed no significant deviation from the form that has been found for VITX materials having smaller pore diameters.[13] Differences between the thermodynamic distribution coefficient and the value of K determined by chromatography, as should show up in the case of hydrodynamic fractionation, could have been proven by a comparison of the value of K determined by a batch and by the chromatographic experiment. Here, however, only the peak broadening will be checked for indications of hydrodynamic separation.

In order to simplify the evaluation, the relation between the variance μ_2 of the elution curve ($=HV_e^2/L$, where L is the length of the column) and the volumetric flow $\dot{V}$ was examined rather than the H–u functions. The μ_2–$\dot{V}$ relations can be represented quite well by regression lines (Table I). The slopes of the μ_2–$\dot{V}$ relation determined for the VITX-120120 column were considerably smaller than the slopes of samples that were not totally excluded at other VITX columns (compare Table II in ref 13). The low absolute values of the dispersion and their small dependence on the flow rate at the VITX-120120 column give a strong indication of the influence of hydrodynamic transport (Figure 1).

In the case of the CPG material, which has a greater mean particle diameter, a stronger dependence of broad-

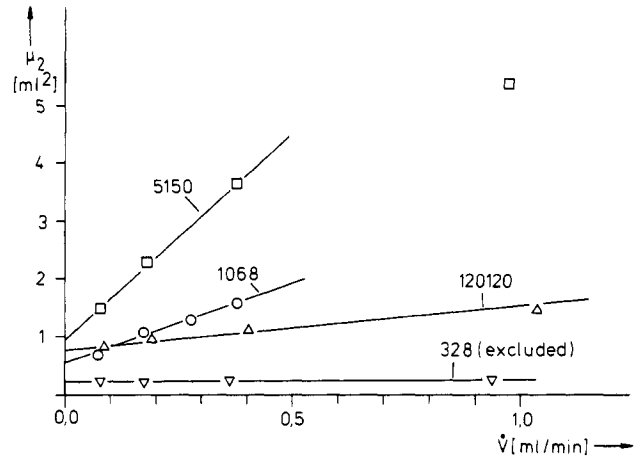

Figure 1. Dispersion as a function of flow rate for standard 5 at different VITX columns.

Table II
Values of qd_p^2/γ Calculated from the Slopes Given in Table I

column	standard	qd_p^2/γ, μm^2	column	standard	qd_p^2/γ, μm^2
VITX	2	52	CPG	2	128
	3	42			
	4	22		4	130
	5	17			
	6	13		6	68
	7	13		7	52
	8	5		8	32
	9	3		9	28

ening on flow rate was found (Table I), although it was much less than would have been expected from the results on the columns VITX-328 to VITX-5150. As distinguished from the behavior at the VITX column, the extrapolated value of μ_2 at $\dot{V} = 0$, which for samples of lower molecular mass lies but little above the values at the VITX columns, strongly increases with increasing molecular mass.

If at both columns investigated the transport in the internal pores is mainly accomplished by flow, according to eq 7 the factor qd_p^2/γ (where γ is the obstruction factor for the diffusion in the pores, $D_s = \gamma D$; compare ref 13) should be independent of the sample or, if the obstruction increases with decreasing steric exclusion, should decrease with decreasing molecular mass.

The calculation of D was accomplished by using the relations given in ref 13. As at both columns no samples were totally excluded, V_0 and KV_i could only be estimated, using the mean value of V_0 for the columns VITX-328, -1068, and -5150. It is 1.22 mL and lies sufficiently below the elution volume of the high molecular mass standard 9 ($\bar{M}_n$ = 773 000), which was 2.25 mL for the VITX-120120 column and 2.50 mL for the CPG column. The relative error in KV_i made by this estimate of V_0 should thus be small.

For comparison with the calculated values of qd_p^2/γ (Table II) the corresponding mean values for the VITX materials with smaller pores are quoted.[13] They are 404 μm^2 for VITX-1068 and 351 μm^2 for VITX-5150. The values in Table II are all significantly lower. They show a decrease with increasing molecular mass of the sample and are most distinct in the case of the VITX column. The slopes of the μ_2–$\dot{V}$ relation are thus much less than would be expected from eq 7. This is also the case for the absolute values of dispersion. For example, at a value of qd_p^2/γ of 300 μm^2 for sample 9 at a flow rate of 1.0 mL/min a permeation dispersion of 0.325 mL^2 would be expected. The overall dispersion found at this flow rate was only 0.072 mL^2.

These calculations confirm the supposition that at both columns investigated the permeation dispersion is mainly controlled not by diffusion but by flow of the macromolecules in the internal pores of the packing.

We now investigate whether the μ_2–$\dot{V}$ relations found also satisfy eq 11 and 13 quantitatively.

First the results obtained at the VITX-120120 column are discussed. With pure convective transport in the range of flow rates studied the value of μ_2 extrapolated on $\dot{V}$ = 0 should decrease with smaller values of V_e (eq 11). Actually the extrapolated values increase, while the slope decreases. These facts indicate the possibility that in the range studied the μ_2–$\dot{V}$ relation is not strictly linear, i.e., that a changeover from diffusional to convective transport still takes place. The range of changeover seems to be broader than indicated by eq 13, possibly because of the action of additive broadening effects in the internal pores, as described in the theoretical section, and of the irregular shape of the packing.

Nevertheless, for a rough estimate we start from the assumption that the permeation dispersion of standard 9 is determined mostly by flow transport, which is only accompanied by a small increase of μ_2 with $\dot{V}$ due to dead-end pores and adsorption effects. The value of μ_2 extrapolated to $\dot{V} = 0$ then contains the polydispersity broadening, the broadening in the interstitial volume, and the broadening due to convective transport in the internal pores. The total plate height at $\dot{V} = 0$ is 1.18 mm. If one neglects the polydispersity broadening and takes as H_{mob} the lowest value that has been found with excluded samples at VITX columns with smaller pores (H_{mob} = 0.44 mm for the VITX-5150 column), one gets for H_{perm}^{flow} the maximum value of 0.74 mm. For the calculation of a theoretical value of H_{perm}^{flow} from eq 11, we approximate the interstitial and the internal pores by cylinders with radii r_i and r_s. If one neglects the difference between u_s and u_s', $u/u_s' = (r_i/r_s)^2$. $d_p \approx 40$ μm and r_g is approximated with 13 μm ($^1/_3 d_p$). The hydrodynamic radius of the internal pores might be about twice as great as the radius determined by Hg porosimetry;[14] i.e., $r_i \approx 0.2$ μm. With V_e = 2.25 mL, $V_0 \approx 1.22$ mL, and $KV_i \approx 1.03$ mL, one gets from eq 11 H_{perm}^{flow} = 82 mm. The experimentally determined maximum value of H_{perm}^{flow} thus lies about 2 orders of magnitude below the value expected from eq 11. As has been calculated above, for pure diffusional transport in the internal pores of VITX-120120 a value of μ_2 = 0.325 mL^2 would have been expected at a flow rate of 1 mL/min; that means that H_{perm}^{diff} = 32 mm. According to eq 12, the changeover from diffusional to convective transport should occur for this high molecular mass standard at about this flow rate.

Actually even standards with much smaller molecular mass show a significant influence of convective transport. It seems that the ratio u/u_s' is considerably smaller than supposed in the approximation used.

The CPG material has a pore diameter which is greater by a factor of 1.5. The particle size distribution is larger and its mean value is greater. d_p is assumed as 60 μm and r_g as 20 μm. According to eq 13 the changeover from diffusional to convective transport should occur somewhat earlier than in the case of column VITX-120120 and the value of H_{perm}^{flow} should be greater by a factor of 1.5.

Actually, however, the CPG material shows strong indications that the changeover is not yet complete in the range investigated and that the limiting value of H_{perm} lies significantly higher. The slope cannot be accounted for by the adsorption at the unmodified material, as this effect

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_{perm}^{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

$$V_e = V_0 + KV_i \qquad \text{where } 0 \le K \le 1 \qquad (1)$$

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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