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OPTIMIZATION OF EFFICIENCY IN SIZE-EXCLUSION CHROMATOGRAPHY

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SUMMARY

The optimization of resolution in size-exclusion chromatography and, hence the accuracy of molecular-weight distribution measurements can be achieved either by increasing the pore volume in the column or by increasing the column efficiency. The first way is limited by nature, because a high pore volume is associated with limited pressure stability. Consequently, improvement must be achieved by increasing the column efficiency by reducing the particle diameter. Small particle diameters of *ca.* 3 μm are advantageous, because it is then possible to work at the minimum of the *h vs. u* curve at usual liquid chromatographic velocities of *ca.* 3 mm/sec, thus achieving a high speed of analysis. The problems usually associated with the use of particles of such a diameter are negligible in the size-exclusion mode. Because sample size is not usually a problem, larger column diameters can be used. Therefore, good standard instrumentation is sufficient for highly efficient size-exclusion chromatography with small particles.

INTRODUCTION

The separation potential of size-exclusion chromatography (SEC)[1,2] is limited. Separation takes place only in the pore volume, V_p, of the stationary phase within the column and should be finished (by definition) with the elution of the smallest molecule, usually the eluent molecule. The latter corresponds to the volume of the mobile phase, V_m, within the column, the starting point of sorptive chromatography. Considering these limitations of resolving power, the main emphasis in practical and theoretical work in SEC has been on describing and minimizing peak broadening[3–8]. In addition to these factors, which determine peak dispersion in liquid chromatography and are treated by different approaches, like the Van Deemter, Golay, and Knox equations, in SEC peaks are dispersed by additional, superimposed mechanisms: the solutes differ not only in their average molecular masses, but consequently also in their diffusion coefficients (up to a factor of 100 and even more); the solutes differ in the time they spend within the pores due to partial exclusion, and last but not least the polymeric solutes are separated according to size during their passage through the column, apparently additionally dispersed.

Therefore, in SEC one must differentiate between peak dispersion caused by

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the properties of the chromatographic system (particle diameter, eluent flow, viscosity, etc.), and peak broadening caused by a partial separation of the polydisperse polymeric solutes. Different approaches have been used to distinguish these processes, *e.g.*, by the reversed-flow method of Moore[9] where band-broadening caused by polydispersity can be eliminated but not that due to differences in diffusion coefficients. In this paper, band-broadening as a function of molecular weight (diffusion coefficient) and accessibility of pore volume is described by using monomolecular polymeric solutes, isolated by sorptive chromatography from SEC polystyrene standards.

Optimization of SEC can be achieved not only by minimizing band-broadening (see above) but also by increasing the pore volume, V_p, within the column and by reducing the dead-volume, the interstitial volume, V_z, within the column. Silicas now widely applied in SEC as such or as chemically surface-modified stationary phases can be prepared with different specific pore volumes and pore diameters, and consequently with different specific surface areas[10]. For chromatographic purposes, the specific values are less important than the values related to the column volume. In the following, these volumes, related to the volume of the empty column, defined as the pore porosity $\varepsilon_p = V_p/V_k$ (where V_k is the empty column volume) and the interstitial porosity $\varepsilon_z = V_z/V_k$ will be used exclusively. The sum of both, the total porosity ε_t can be simarly calculated from V_m. It has been shown that doubling the specific pore volume leads to only a 30% increase in pore volume within the column[11]. Additionally, nature limits this approach, because silicas with large specific pore volume are like sponges[12] and cannot be used under conditions approaching high-performance liquid chromatography (HPLC). However, as will be demonstrated in this paper, by optimizing the $\varepsilon_p/\varepsilon_z$ relationship the peak capacity of SEC can be additionally improved.

EXPERIMENTAL

Instrumentation

The equipment was assembled from commercially available and laboratory-built units. Special attention was paid to keeping the extra-column band-broadening small. The detector cell volume was reduced to 4 μl. A Waters 6000 A pump, was used. Flow-rates of less than 0.1 ml/min were achieved by using the Waters Model 660 gradient programmer. Columns were made from drilled-out stainless steel tubes and modified Swagelok fittings. V_k was determined volumetrically. The columns and the eluent were thermostatted to 25°C.

Stationary phases and eluents

Silica "250 A/HPLC" (Grace, Worms, F.R.G.) (particle diameter, d_p = 3, 5, 7 and 10 μm), Nucleosil 100 and 100 V (Machery & Nagel, Düren, F.R.G.) (d_p = 5 μm), Zorbax ODS (DuPont, Wilmington, DE, U.S.A.) (d_p = 7 μm) and a laboratory-prepared reversed-phase packing (H90/10) d_p = 10 μm were used. The properties of these materials are summarized in Table I. Bonded phases (RP C_{18} and Amide) were prepared, as described earlier[13,14]. The particle diameters were determined by a "Coulter Counter", Model TA II (Coulter Electronics, Hialeah, U.S.A.) and from chromatographic data[15].

As eluent for SEC with polystyrenes, dichloromethane (distilled) was used ex-

TABLE I

PROPERTIES OF THE STATIONARY PHASES

Phase	*Particle diameter (μm)*	*Specific surface area (m^2/g)*	*Pore diameter (nm)*	*V_p (ml/g)*	*k^*_{max} (c_p/c_z)*
250 A/HPLC	3, 5, 7, 10	300	15	1.7	1.40
Nucleosil 100	3.3, 5	300	10	1.2	1.15
Nucleosil 100 V	5	380	10	1.5	1.46
H 90/10 RP	10	400	6	0.5	0.6
Zorbax ODS	7	300	< 5	0.2	0.4

clusively. With this eluent, polystyrenes are not retarded on either silica or C_{18} stationary phases. The amide phase was used with aqueous buffers for protein analysis.

Column packing

Columns were packed by a standardized method[16] a combination of the "viscosity" and "balanced-density" methods with isopropanol–cyclohexanol (3:1, v/v) for bonded phases, or with isopropanol–carbon tetrachloride for silica materials. The composition had to be adjusted to the porosity of the materials. Materials with a large specific pore volume (Grace 250A/HPLC and Nucleosil 100 V) had to be packed carefully. These fragile materials were packed at low starting flow-rates to minimize shear forces. The lower column fraction (*ca.* 5 cm) was removed and discarded[16]. The maximum packing pressure was 420 bar, and the maximum flow-rate 6 ml/min.

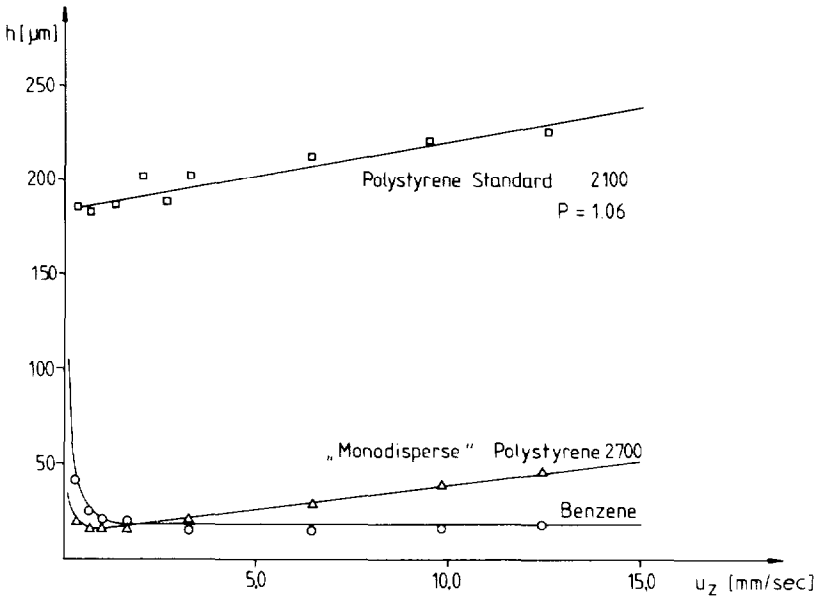

Fig. 1. Band-broadening of polystyrene standards. Column: Nucleosil 100 V, d_p = 3 μm, 125 × 4.2 mm I.D. Eluent: dichloromethane. Solutes: polystyrene standard 2100, polydispersity (P) = 1.06; "monodisperse" polystyrene MPS 2700; benzene.

Solutes

Polystyrene standards were obtained from Pressure Chemical Co., Pittsburgh, PA, U.S.A. Their standard 2100 was separated into its oligomers by reversed-phase (RP) chromatography (21% C (w/w) RP H90/10; 10 μm; gradient from methanol–dichloromethane (9:1) to methanol–dichloromethane (6:4) in 20 min). In addition to the separation into the individual oligomers, different isomers (diasteromers) were observed by the increasing peak broadening, which caused overlapping with the adjacent oligomer peaks. Therefore, a pure homomolecular solute, consisting of a single molecular species, could not be obtained. The highest-molecular-weight oligomer (mol.wt. = 2700) so prepared contained less than 20% of the preceding and following homologues. Other homologues than $n-1$ and $n+1$ could not be observed in this preparation by isocratic RP chromatography.

The advantages of such "monodisperse" polystyrenes in the discussion of peak broadening in SEC can be seen in Fig. 1. The smallest plate height (h) obtained for the commercial polystyrene standard, PS 2100, is larger by a factor of 20 than that of the "monodisperse" standard PSM 2700.

RESULTS AND DISCUSSIONS

Peak broadening in SEC with "monodisperse" polystyrenes

In liquid chromatography, peak broadening is a function of the linear velocity of the eluent, the particle size of the stationary phase, and capacity ratio, k', of the solute. Generally, differences in diffusion coefficient can be neglected, because the molecular masses of the solutes separated are similar and usually below 500. In contrast, in SEC the molecular masses, and hence the diffusion coefficients, differ widely within a sample. Their influence on peak broadening must also be considered.

The fraction of the pore volumes accessible to the solutes decreases with increasing molecular mass. This also influences band-broadening. In SEC, the analogue to the capacity ratio k' in sorptive chromatography is the ratio of the probabilities k^* of the sample staying in the stagnant mobile phase inside the pores or in the moving mobile phase between the particles (*i.e.* the interstitial volume V_z). In SEC, the k^* value is limited and its maximum value is given by the ratio of the pore volume and the interstitial volume of a column or the pore porosity ε_p and the interstitial porosity ε_z

$$k^*_{max} = \frac{V_p}{V_z} = \frac{\varepsilon_p}{\varepsilon_z}$$

The maximum k^* value is governed by the physical properties of the stationary phase and by the column-packing procedure. The linear velocity, u_m, measured by the dead-time, t_m, is the velocity of the eluent molecules averaged for the velocity within the pores (linear velocity, $u = 0$) and that between the particles, the interstitial velocity, u_z. The error introduced by conveniently measuring the eluent velocity via t_m is constant, as long as the porosities of the stationary phases and the packing procedures are similar. In SEC, the porosities of the stationary phases must be different, and therefore the eluent velocity must be described in terms of the interstitial velocity[17].

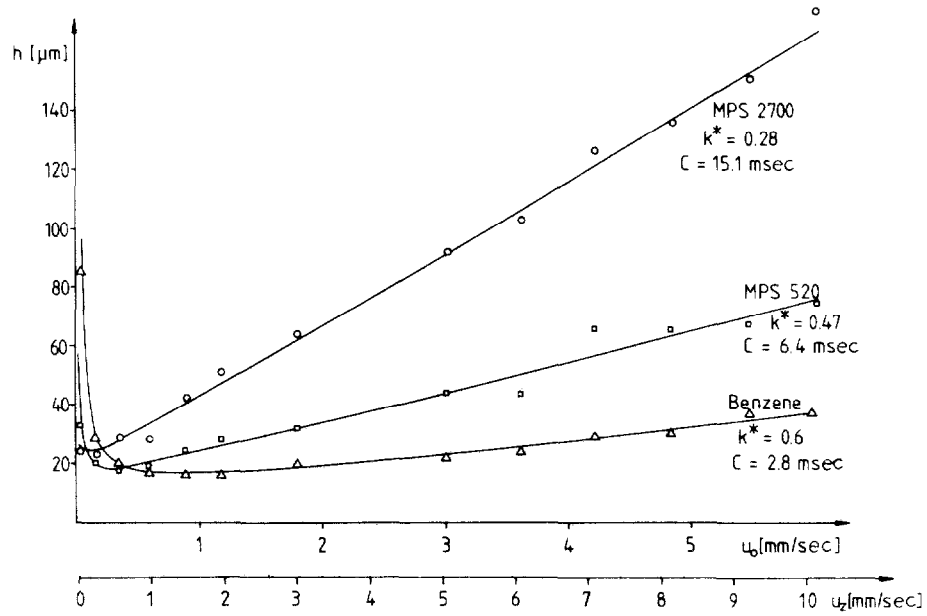

Fig. 2. Band-broadening with "monodisperse" polystyrene. Column: d_p = 10 μm, pore diameter 6 nm, 250 × 4.2 mm I.D. H 90/10 RP. Eluent: dichloromethane. Solutes: benzene, MPS 520 and 2700.

Three typical *h vs.* u_z curves, obtained with different silicas, will serve to demonstrate the influence of diffusion coefficient D_m, particle diameter, k^* value, and interstitial velocity on column performance. Fig. 2 shows the *h vs. u* curve obtained with 10-μm silica with a k^*_{max} value of 0.61. As solutes, "monodisperse" polystyrenes (PS) with molecular weights 520 and 2700 were used. As expected, the *C* term increases with increasing molecular weight from 2.8 msec for benzene ($k^* = k^*_{max} = 0.61$) to 6.4 msec for PS mol.wt. 520 ($k^* = 0.47$) and to 15.1 msec for PS mol.wt. 2700 ($k^* = 0.28$). With increasing molecular mass (increasing diffusion coefficients) maximum efficiency is achieved at lower velocities, as predicted by the Van Deemter equation[4,18].

Fig. 3 shows the *h vs. u* curves for a 7-μm stationary phase with an extremely small k^* value of 0.4. Also, in this case the *C* term rises with increasing molecular mass from 0.9 msec for benzene ($k^* = k^*_{max} = 0.4$), to 4.5 msec for PS mol.wt. 520 ($k^* = 0.24$), to 8.4 msec for PS mol.wt. 1050 ($k^* = 0.18$), and to 11.3 msec for PS mol.wt. 2700 ($k^* = 0.1$). In the last case, the *C* term was calculated only for the linear region of the plot. The curve is atypical, probably owing to the very small k^* value and, hence, a hindered diffusion in and out of the pores. This stationary phase has not be designed for SEC, but mainly for sorptive chromatography. Owing to the smaller particle diameter the *C* terms are generally smaller than in the previous example.

With a 3-μm stationary phase, still smaller *C* terms can be obtained, as shown in Fig. 4. With benzene ($k^* = k^*_{max} = 1.2$) a *C* term of 0.49 msec, and with PS mol. wt. 2700 ($k^* = 0.8$) one of 2.9 msec was obtained. As can be seen, the minima of the *h vs. u* curves are again shifted to very small linear velocities, relative to that of benzene, the inert solute in sorptive chromatography.

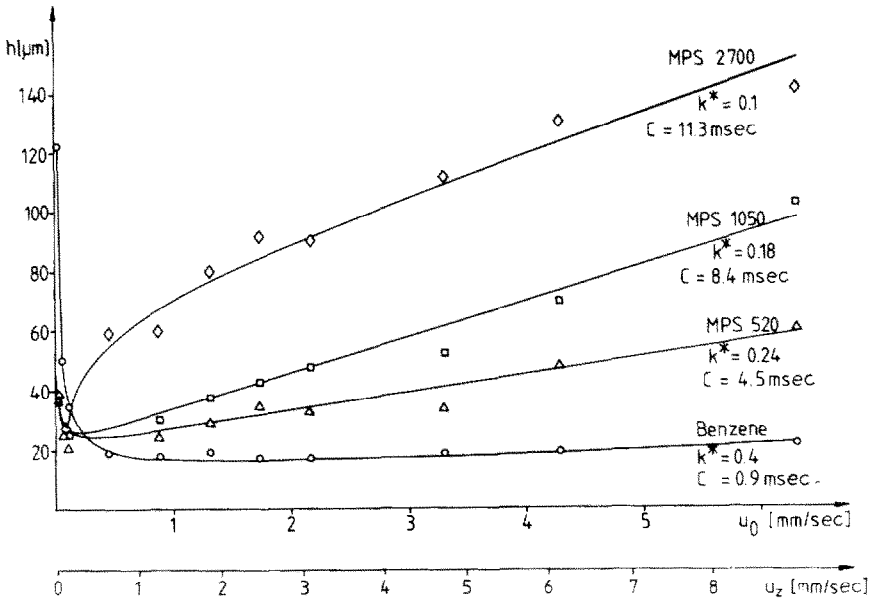

Fig. 3. Band-broadening with "monodisperse" polystyrenes. Column: Zorbax ODS, d_p = 7 μm, pore diameter < 5 nm, 250 × 4.2 mm I.D. Eluent: dichloromethane. Solutes: benzene, MPS 520, 1050 and 2700.

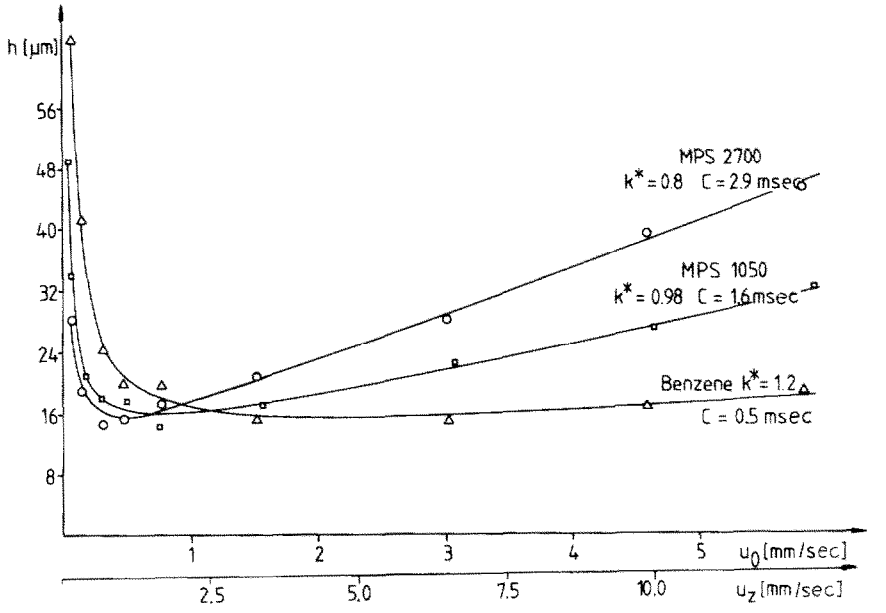

Fig. 4. Band broadening with "monodisperse" polystyrenes. Column: Nucleosil 100, d_p = 3.3 μm, pore diameter 10 nm, 125 × 4.2 mm I.D. Eluent: dichloromethane. Solutes: benzene, MPS 1050 and 2700.

The evaluation of the different h *vs.* u curves for twelve different stationary phases allowed the determination of the constants of the van Deemter equation. The average value of the constant of the A term was 3.5 ± 1.5, that of C term (0.6 ± 0.3) k^*. For the B term a constant (1.3 ± 0.9) (1 + k^*) was calculated. This value could not be determined with the same accuracy as the other values, because, owing to the required low eluent velocities, the number of data points is smaller.

For the description of the h *vs.* u curves, several equations have been derived[6,18–20], which also allow the description of peak-broadening phenomena in SEC. However, the C terms calculable with these equations for monodisperse polymers are much smaller than the measured ones. Halász *et al.*[21] derived from the Van Deemter[18] and Golay[22] equation a simplified empirical version, which allows the description of the peak broadening of inert solutes in HPLC as a function of solute diffusion coefficient, eluent velocity, and particle diameter. The inert solute in HPLC is the last component to be eluted in SEC. Consequently, it can be postulated that this equation can also be used to describe peak broadening in SEC. The empirical version of this equation with the thus determined constants, extended to SEC (using u_z instead of u_m and introducing k^*) then reads

$$h = 3.5\, d_p + 1.3 \frac{(1 + k^*)D_m}{u_z} + 0.6 \frac{k^*}{(1 + k^*)^2} \frac{d_p^2}{D_m} u_z$$

The main difference between this equation and those derived from the rate theory is that here the C term is larger by a factor of 20. Of course, this equation also contains the assumption that the peak broadening in the interstitial volume is constant. It is difficult to prepare "monodisperse" polystyrene standards with molecular masses above 3000 and to obtain experimental data on peak broadening in this region of molecular masses. However, it can be assumed that the peak-broadening of polymers with molecular masses above 3000 can be described with the same equation, at least for polystyrenes with dichloromethane as eluent, if the appropriate diffusion coefficients are applied. In Fig. 5, the calculated peak-broadening is shown

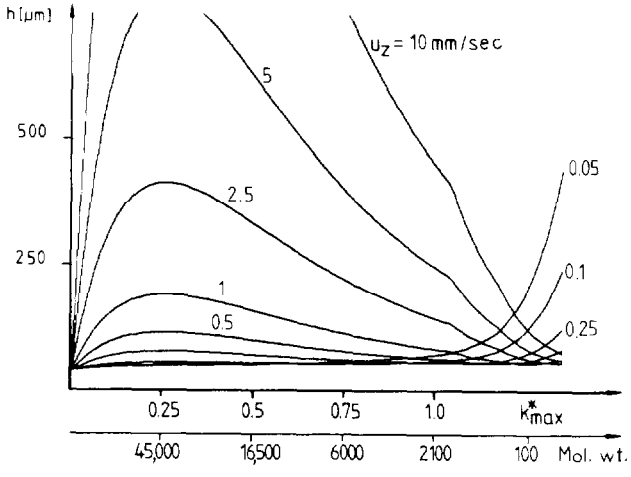

Fig. 5. Band broadening as a function of flow-rate and molecular mass (calculated h values for d_p = 10 µm and k^*_{max} = 1.35).

as a function of the molecular mass, *i.e.* as the probability ratio k^* of a molecule staying in the pores or in the interstitial volume, for different velocities. As average particle diameter, 10 μm was used. The diffusion coefficients were calculated from the molecular masses, obtained by linearizing the calibration curve (log mol.wt. *vs.* k^*) of the silica used. For this purpose, a maximum k^* value of 1.35 was used, the maximum value obtained in packing a silica with a specific pore volume of 1.7 cm^3/g under standard conditions.

At a linear velocity of 10 mm/sec., h values of *ca.* 1000 μm for k^* values of 0.25 were calculated. This enormous peak-broadening can be significantly reduced by reducing the linear velocity. With the same stationary phase and linear velocities below 0.25 mm/sec, the peak-broadening in the region of high molecular masses (small k^* values, small diffusion coefficients) decreases, and acceptable h values are obtained.

With the low-molecular-weight species, the h values are also decreased by decreasing the linear velocity. However, at velocities below 1 mm/sec peak-broadening increases again, owing to the increasing influence of the longitudinal diffusion (B term). For benzene at a linear velocity of 0.05 mm/sec, an h value of 350 μm can be calculated and measured.

Decreasing the particle diameter, of course, reduces the peak broadening of solutes with high molecular masses. The maximum h values at $k^* = 0.25$ decrease from over 1000 μm to 300 μm for 5-μm particles and to 120 μm for 3-μm particles. Because the B term is independent of the particle diameter, the h values at the low molecular mass side are not reduced. On the contrary, because the minimum of the h *vs.* u curve is shifted to higher linear velocities when the particle diameter is reduced[23], the influence of the B term on peak broadening becomes more and more important. Therefore, it is necessary to determine the optimal linear velocity for the separation range of a column. This velocity is a function of the diffusion coefficient of the solute (k^* value, molecular mass) and of the particle diameter. Under optimum conditions, the average peak-broadening of solutes with high as well as with low molecular weight is minimal.

Optimum conditions in SEC

The separation capacity in SEC is limited by the porosity of the stationary phase. As discussed, peak broadening varies strongly within a separation as a function of accessibility to the pores (k^* value) and of the diffusion coefficient. The maximum number of peaks that can be separated is, consequently, a function of the relationship of the pore volume within the column and the peak volume, determined by peak-broadening of the "monodisperse" solute. The number of peaks that can be separated within a given column (porosity of the stationary phase, particle diameter) can be used to characterize optimum separating conditions in SEC. Of course, the separation of single peaks is very seldom the aim of SEC. More often, the determination of the polydispersity or the molecular-mass distribution of a polymeric solute must be achieved. The accuracy of these analysis increases with increasing peak capacity of the SEC system. (The term peak capacity, introduced in sorptive chromatography by Giddings[24], cannot be transferred to SEC without alterations. In sorptive chromatography, where the diffusion coefficients are very similar within an analysis, peak capacity is always proportional to the square root of the plate number n of a column.)

To demonstrate the influence of particle diameter and linear velocity on the separation capacity in SEC, the number of peaks that can be separated with a resolution $R = 1$ was calculated. The peaks are represented as triangles with a base of 4σ. This peak width was obtained by using the modified Van Deemter equation, derived above, with the experimentally determined constants for the A, B and C terms. The calculations were performed by iteration to determine the elution volume of the next peak and its molecular mass from the calibration curve, thus its diffusion coefficient and, consequently, peak-broadening, etc. for linear velocities between 0.01 mm/sec to 10 mm/sec. The results are based on columns, packed with 10-μm and 3-μm silica gel (Grace 250 A/HPLC) with a maximum k^* of 1.4 for the separation of polystyrenes in dichloromethane. In Fig. 6 the separation capacity of 10-μm and 3-μm particles in SEC are compared. With the 10-μm particles at high linear velocity, peak broadening due to the large C term is high. However, only five peaks can be separated, within an analysis time of less than 1 min. With decreasing linear velocity, the peak broadening of all peaks within the separation range decreases. At a linear velocity of 1 mm/sec, eleven peaks can be separated in less than 10 min. Below linear velocities of 1 mm/sec, the peak broadening of the high-molecular-mass solutes still decreases, whereas for the low-molecular-mass solutes peak broadening increases, but the separation capacity still increases. At very low velocities the number of peaks that can be separated decreases because more additional solutes are influenced by the longitudinal diffusion term. The optimum velocity for 10-μm particles is *ca.* 0.05–0.1 mm/sec, resulting in analysis times of 90 to 180 min with 25-cm columns.

A qualitatively similar relationship can be seen with 3-μm particles, but the individual terms of the Van Deemter equation influence separation efficiency differently. The great advantage of these small-particle columns can be seen very clearly. With an analysis time reduced by a factor of 100, the 3-μm column at a velocity of 10 mm/sec permits the separation of as many compounds as can be separated with a 10-μm column under optimum conditions. However, even with 3-μm particles, at this velocity the C term noticeably influences peak dispersion. With decreasing linear velocity, peak broadening of the high-molecular-mass solutes decreases, but the low-molecular-mass solutes are very soon influenced by the B term. Maximum separation efficiency is achieved, therefore, at higher linear velocities around $u_z = 0.5$ mm/sec, resulting in an overall analysis time of 20 min. Of course, the separation capacity is doubled when the particle diameter is reduced from 10 μm to 3 μm. Decreasing the particle diameter from 10 μm to 3 μm and working under optimal conditions yields

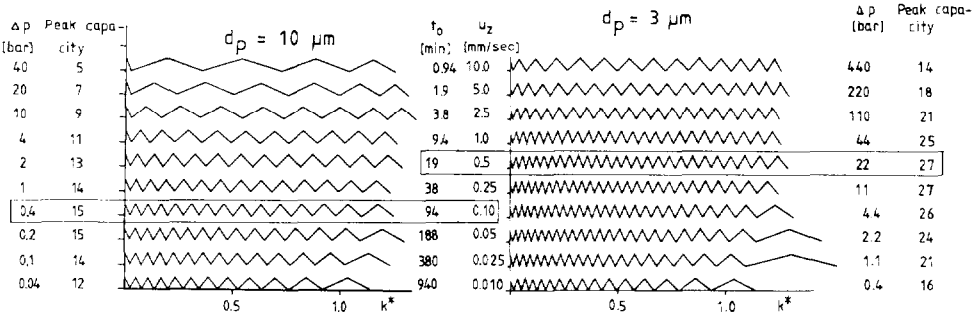

Fig. 6. "Peak capacity" in SEC. Demonstration of the influence of particle diameter and flow-rate on separation efficiency and analysis time.

a duplication of the separation efficiency and reduces analysis time by a factor of 5.

Columns packed with 5-μm particles are between these limits and behave similarly. Under optimum conditions, *ca.* 20 peaks can be separated, at an optimum velocity *ca.* 0.25 mm/sec in an analysis time of *ca.* 30 min.

These discussions on peak capacity demonstrate, of course, how the chromatographic parameters of optimum particle diameter and optimum flow-rate resulting in minimum kinetic peak dispersion can be adjusted for optimum conditions in SEC.

Porosity of the stationary phases

In addition to the kinetic effects discussed so far, the porosity of the stationary phase, especially the relationship of pore porosity and interstitial porosity, influences the separation capacity of and the analysis time with a stationary phase. A large separation capacity means increased accuracy of determination of molecular mass distribution. Small interstitial porosity, on the other hand, means shorter analysis time, because the time needed for the elution of a totally excluded peak means lost (dead) time from the point of view of an analyst. With the classical swollen gels, such as Sephadex, this relationship could approach values up to two. With rigid materials, such as silica, values below or around one can usually be reached.

There are two possible ways to increase the ratio of ε_p and ε_z. Larger specific pore volumes of the silica result only to a certain extent in an increase of the pore porosity[11]. Nature limits this possibility, because with increasing specific pore volume the mechanical (pressure) stability of the silica decreases.

By varying the packing procedure, it is possible to decrease the interstitial porosity[16]. For irregular silicas, values of ε_z between 0.42 to 0.52 have been achieved. With spherical silicas, especially developed for HPLC, values of ε_z as low as 0.35 could be measured[25]. However, owing to their preparation, the pore porosities of these materials are low. Depending on the shape of the silicas and their specific pore volume, values of $\varepsilon_p/\varepsilon_z$ between 0.6 and 1.45 could be achieved. The latter, largest value was obtained with a silica having a specific pore volume of 1.7 ml/g, which was rounded to spheroidal shape during the grinding and classification procedure.

From this point of view, an optimal stationary phase for SEC would be a pressure-stable rigid material (silica) with a large specific pore volume (large ε_p) of spherical shape (small ε_z), available as narrow size range fractions of small particle diameters (5 μm or, better, 3 μm), and with different average pore diameters up to 500 nm, or even larger.

Demonstration of separation efficiency

The influence of porosity and efficiency on separation in SEC are demonstrated with three sample chromatograms. For this demonstration, "monodisperse" polystyrenes were used. The eluent flow was selected for each individual particle diameter to achieve maximal peak capacity.

Fig. 7 demonstrates the separation of the polystyrenes with a spherical silica, developed for HPLC. This material has a small average pore diameter (less than 5 nm) and an extremely small pore volume. (However, the specific surface area, necessary for sorption chromatography is large —*ca.* 300 m^2/g.) Because of the low ratio of pore porosity to interstitial porosity of 0.4, *ca.* 70% of the entire analysis time is wasted as dead-time. The linear velocity of 0.3 mm/sec, optimal for 7-μm particles,

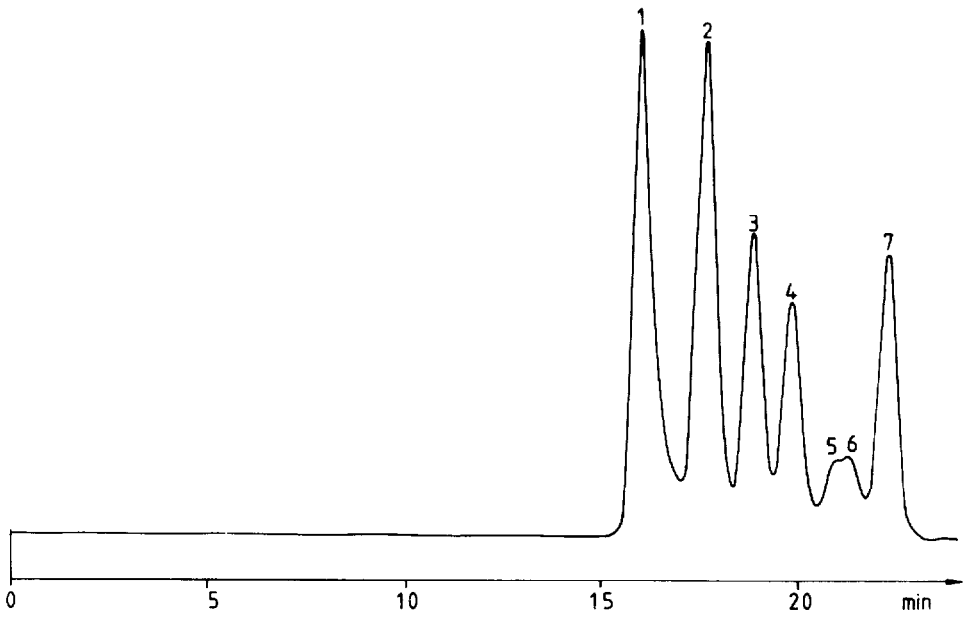

Fig. 7. Separation of polystyrene standards. Conditions as in Fig. 3, u_z = 0.3 mm/sec. Solutes: 1 = PS 402,000; 2 = MPS 2700, 3 = MPS 1050; 4 = MPS 520; 5 and 6 = isomeric distyrenes; 7 = benzene.

leads to a total analysis time of 25 min.

With 10-μm particles, as used in Fig. 8, a lower linear velocity (0.13 mm/sec) must be used to work under optimum conditions, resulting in an analysis time of *ca.* 50 min. Because of the larger porosity ratio of 0.6, the dead-time portion of the total analysis time is smaller than in Fig. 7. Because of the larger pore volume and pore diameter, solutes with a molecular mass above 2700 could have been included in this chromatogram.

The possible high speed of analysis achievable with small particles becomes obvious from Fig. 9. Here, silica with a particle diameter of 3.3 μm was used. Because of the greater efficiency, only half the column length was used. The analysis time of *ca.* 8 min must, of course, be doubled compared with that in Figs. 7 and 8. Because of the large porosity ratio of 1.15, only 40% of the total analysis time is wasted as dead-time. The pore diameter is slightly larger than in the previous example. However, owing to the larger pore porosity of this silica, only 40% of the separation capacity is used with the standards. The problems in optimizing the flow-rate in SEC can also be seen in this example. The flow-rate was optimized for the 2700 polystyrene standard. The influence of the longitudinal diffusion term for the low-molecular-weight solutes can already be seen in the broadened benzene peak.

CONCLUSIONS

By using monodisperse high-molecular-weight polystyrene solutes, the peak broadening in SEC due to kinetic effects could be described in analogy to the dispersion in conventional HPLC. The influence of the kinetic behaviour on the dis-

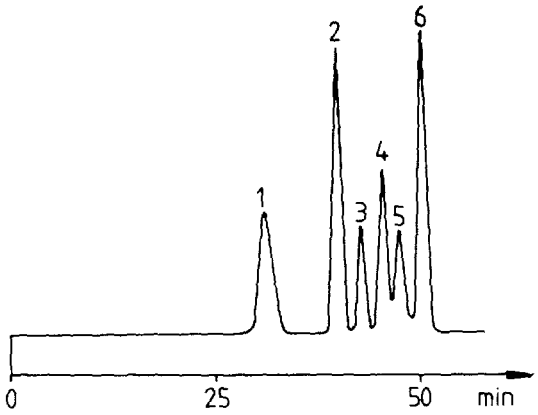

Fig. 8. Separation of polystyrene standards. Conditions as in Fig. 2, u_z = 0.13 mm/sec. Solutes: 1 = PS $3.7 \cdot 10^6$; 2 = MPS 2700; 3 = MPS 1050; 4 = MPS 520; 5 = distyrene; 6 = benzene.

persion on the high-molecular-weight side of the elution profile of a polymer must be discussed independently from the kinetic peak broadening on its low-molecular-weight end. The discussion of the kinetic peak-dispersion, as a function of particle diameter, flow-rate and the molecular weight of the solutes shows that for each particle diameter an optimal eluent velocity exists. This is due to the small diffusion coefficients considerably lower than in sorption column chromatography, where the molecular weights of the solutes rarely exceed 500. The number of peaks that can be separated, or the accuracy of measurement of molecular-weight-distribution curves increases with decreasing particle diameter. But decreasing the particle diameter results —as in sorption chromatography— in an increase of the optimal flow velocity and of the speed of analysis.

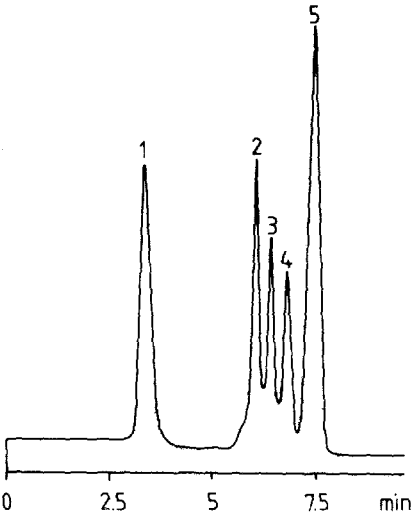

Fig. 9. Separation of polystyrene standards. Conditions as in Fig. 4, u_z = 0.6 mm/sec. Solutes: 1 = PS 402,000; 2 = MPS 2700; 3 = MPS 1560; 4 = MPS 730; 5 = benzene.

Because of the low velocities optimal in SEC compared with conventional chromatography, where solutes with molecular weights *ca.* 300 are usually separated, many problems discussed for chromatography with small particles are negligible in SEC. With 3-μm particles and an optimum velocity of 3 mm/sec of dichloromethane, a pressure drop of 300 bar for a 25-cm column is required. Because sample size generally is not a problem in SEC, wider columns can be used. Therefore, the volume of the detector cell does not cause the problems in SEC that it does in conventional liquid column chromatography with very small particles. A time constant of 0.25 sec, which a good LC detector should have, anyhow, should be sufficient to give undistorted peaks with h values of *ca.* 10 μm, generated with a column length of 25 cm at linear velocities below 1 mm/sec.

From this point of view, the particle diameter of stationary phases could be decreased further. Despite the problems in packing and using columns with particles of, *e.g.*, *ca.* 1 μm, it seems impossible to prepare particles with such a diameter and the pore diameters above 20 nm required for polymers with molecular masses above 500,000. Another compromise in optimizing efficiency, speed of analysis, and separation range will then be required.

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