High Performance Size Exclusion Chromatography with Microparticulate Porous Silica Spheres. Influence of Flow Rate, Salute Mass Transfer and Polydispersity of Samples

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PLEASE SCROLL DOWN FOR ARTICLE

HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY
WITH MICROPARTICULATE POROUS SILICA SPHERES.
INFLUENCE OF FLOW RATE, SOLUTE MASS TRANSFER
AND POLYDISPERSITY OF SAMPLES

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ABSTRACT

The contributions to peak broadening in Size Exclusion
Chromatography with microparticles of porous silica
spheres having narrow size distributions have been de_
termined by measuring the plate height dependence on
flow rate for toluene and for polystyrene standards co_
vering a wide range of molecular weights. From these
contributions, the diffusion coefficients of the macro_
molecules in the pore matrix and the polydispersities
of the samples could be evaluated.It is shown that for
permeating polymers the band broadening is determined
by the eddy diffusion in the mobile phase, by the slow
mass transfer of the solute in the stationary phase
and by the polydispersity of the standards. In proper_
ly packed columns the eddy diffusion term is of minor
importance compared to the other effects, whereas the
solute mass transfer, which is a velocity dependent

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process, can be minimized only at extremely low flow
rates.

INTRODUCTION

Rigid inorganic packings are widely used in modern
Size Exclusion Chromatography (SEC) because of their
superior mechanical and thermal stability and their ca_
pability of withstanding the high pressures involved in
high speed liquid chromatography. Since the efficiency
of the SEC columns greatly increases by decreasing par_
ticle size (1), packing materials with particle avera_
ge size of about 10 μm or less are now produced:25 -30
cm long columns, packed with these microparticulate ma_
terials, show efficiencies several times higher than
those of conventional SEC columns (2, 3).

Frequently, if not exclusively, the inorganic mate_
rials used in high performance SEC are porous silica
gels. The determination of the molecular weights of po_
lymers generally improves by decreasing the eluent flow
rate: the situation is less critical when polymer sam_
ples of relatively broad molecular weight distribution
are analyzed (accurate molecular weights have been ob_
tained (4) with a flow rate of 0.5 cm^3 min^{-1}), whereas
with more narrowly distributed polymer samples one has
to resort either to extremely slow flow rates (5), or
to a suitable correction for peak spreading (4).

The flow rate dependence of the efficiency in SEC
with short columns filled by silica microspheres of
narrow size distribution and mean diameter 20 μm and

8 μm was investigated by Dawkins and Yeadon (6, 7),
who were succesful in separating the different contri_
butions which are operative in broadening the chromato_
graphic peaks. Similar results were obtained with a dif_
ferent procedure by Knox and McLennan (8) who pointed
out that in polymer size exclusion analysis with high
performance columns the peak broadening is determined
by the polymolecularity of the sample, which is the pre_
vailing factor, and by a kinetic, flow rate dependent,
contribution.

In the present paper the procedure suggested by
Dawkins (6) to assess the importance of the different
contributions to peak broadening and to determine the
optimum working conditions is applied to packings of
LiChrospher, a porous silica gel produced by E. Merck,
Darmstadt, Germany, which has been found valuable in
giving high resolutions in SEC, and which has already
been the object of an investigation of one of us (4) on
the use of it in molecular weight determinations by SEC.
The present study has been performed on microparticles
(about 10 μm) of different mean pore size, using poly_
mer samples covering a wide range of molecular weights.

PEAK BROADENING IN SEC

In a properly arranged SEC system in which extraco_
lumn effects, deriving from injection, capillary connec_
tions, detector, are minimized the peak broadening pri_
marily depends on processes occurring in the column,
and is a direct consequence of the slow rate of achie_

vement of solute equilibrium between the mobile and the
stagnant phase. For macromolecules the time necessary
to reach this equilibrium can be very long, so that lo_
cal departures from equilibrium give raise to broadening
and asymmetry of the peaks.

Peak broadening in chromatography is adequately ex_
pressed in term of the number N of plates of the column,
and of the plate height H which can be defined as the
peak variance per unit length of the column (9):

$$H = \sigma^2 / L \qquad (1)$$

where σ is the peak standard deviation and L is the
column length. For an experimental chromatogram the pla_
te height is calculated from the relations:

$$H = L / N \qquad (2)$$

$$N = 5.54 \ (V_e / w_{\frac{1}{2}})^2 \qquad (3)$$

where V_e is the elution volume at the maximum of the
peak and $w_{\frac{1}{2}}$ is the peak width at half height.

The theory of chromatography gives relationships
in which the plate height is expressed in termsof sums
of different contributions (10). From this general tre_
atment the theory of peak dispersion in SEC was formu_
lated by Giddings and Mallik (11) and applied to some
experimental data (12), showing the reliability of the
classical chromatographic approach to investigate even
in the SEC case the peak broadening factors.

The dependence of plate height on the solute dis_
persion mechanism in SEC was subsequently investigated
by Dawkins and Yeadon (5,6), who took into account the
contribution from polydispersity in the elution of per_
meating polymers. In this case, the experimental value
of H is given by

$$H = A + \frac{B}{u} + Cu + (\sigma_M^2 / L) \qquad (4)$$

where u is the eluent flow rate, A, B and C are coeffi_
cients related to different diffusion processes of the
solute molecules, and σ_M^2 / L is the contribution of the
solute polydispersity (σ_M^2 is the peak variance depen_
ding on the molecular weight distribution). When stan_
dard polymer samples with low dispersity are eluted,
assuming that their molecular weight distribution may
be represented by a logarithmic normal one, the polydi_
spersity term of eq. (4) takes the form:

$$\frac{\sigma_M^2}{L} = L \frac{\ln (M_w/M_n)}{D_2^2 \, v_e^2} \qquad (5)$$

where D_2 is the slope of the SEC calibration curve, ob_
tained by plotting ln M vs. V_e.

According to Giddings (10), the coefficients A, B
and C of eq. (4) take the form:

$$A = 2 \lambda d_p \qquad (6)$$

(where λ is a constant characteristic of the packing
and d_p is the particle diameter),

$$B = 2 \gamma D_m \qquad (7)$$

(where D_m is the diffusion coefficient of the solute
in the mobile phase, corrected by the obstruction fac_
tor γ),

$$C = q\ R(1-R)\ \frac{d_p^2}{D_s} \qquad (8)$$

(where q is a configuration factor depending on the par_
ticles' geometry, R is the retention ratio V_o/V_e between
the interstitial volume of the column and the elution
volume, and D_s is the solute diffusion coefficient in
the pores).

By introducing eqs. (5), (6), (7) and (8) into eq.
(4) one obtains:

$$H = 2\lambda d_p + 2\gamma\frac{D_m}{u} + q\ R(1-R)\ \frac{d_p^2}{D_s}\ u + L\ \frac{\ln(M_w/M_n)}{D_2^2\ V_e^2} \qquad (9)$$

which expresses the plate height as a sum of terms, the
fourth of which depending upon the sample polydispersi_
ty, and the other three representing true chromatogra_
phic contributions: the first term (term A) comes from
the eddy diffusion of the solute in the mobile phase,
that is the fact that different solute molecules run

along different paths between the gel particles; the
second term (term B) results from the longitudinal dif_
fusion of the solute in the mobile phase; and the third
(term C) comes from the mass transfer in the stationa_
ry phase associated with the slow diffusion of the so_
lute in the packing pores.

By performing measurements with polymer samples dif_
ferently excluded at different flow rates it is then
possible, by applying eq. (9), to know how H varies and
which factor is prevailing to change the efficiency of
a given packing; the reliability of the packing proce_
dure can also be checked together with the permeability
characteristics of the porous material. Finally, by e_
valuating the last term of eq.(9), the polydispersity
index M_w/M_n of the polymer standards can be calculated.

EXPERIMENTAL

LiChrospher packings with nominal particle size of
10 μ m were supplied by E. Merck (Darmstadt, Germany).
Four different silica gels were used, SI 100 (batch
no. YE604), SI 500 (batch no. YE91), SI 1000 (batch no.
YE645) and SI 4000 (batch no. YE445), having different
mean pore size. The microparticles, examined by optical
microscopy, appeared to have a regular spherical shape.
The densities of the different silica gels were deter_
mined with a picnometer.

The gels were dispersed in methanol, stirred in an
ultrasonic bath, and the slurries were packed according

to the upward technique proposed by Bristow et al. (13)
into stainless steel columns (length: 25 cm; internal
diameter: 0.46 cm) at a pressure of about 10 MPa (1 MPa=
10 bar).

High performance SEC was executed with a dual-pis_
ton diaphragm pump (Orlita DMP-SK 15/3), and an ultra_
violet detector (Zeiss, PM2 DLC, cell volume 8 μl, 260
nm). An injection valve (Rheodyne 7010) with a 10 μl
loop was used to introduce the samples into the columns;
all the connections between injector and column and be_
tween column and detector were made of short low dead-
volume capillary tubing (0.025 cm i.d.).

The mobile phase was tetrahydrofuran (Carlo Erba,
Milano, Italy) stored over KOH for 12 h, refluxed over
CaH_2 and distilled. The samples injected were toluene
(Carlo Erba) and polystyrene standards (ArRo Laborato_
ries, Joliet, Illinois, and Polysciences, Warrington,
Pennsylvania) dissolved in tetrahydrofuran at concen_
trations low enough to avoid concentration effects (14)
on the elution volumes. For toluene and for PS standards
up to about $M = 7 \cdot 10^5$ the sample concentration was 0.1%
(w/v), whereas for the highest molecular weight standards
it was 0.05% (w/v). The stream flow rate was detected
in each analysis by measuring the time for collecting a
given volume of the eluent in a burette connected with
the output of the detector. With this system no short
term flow rate variations were detected. The flow rates
investigated varied from 0.15 to 2.5 cm^3 min^{-1}, corres_
ponding to linear velocities ranging from about 0.03

to 0.6 cm sec^{-1}. Lower steady flow rates could not be
obtained with the pumping system adopted.

The elution volumes of the injected samples were
practically independent of the flow rate, except in a
few cases at the highest velocities with the very high
molecular weight standards. The reproducibility of the
measurements was better than 1%. All the results for V_e
and H are the average of at least three injections.

The characteristics of the packed columns were de_
termined by direct calibration with the PS standards.
For the column packed with SI 4000, however, the inter_
stitial volume V_o could not be determined with an ex_
cluded polymer because even a standard of molecular we_
ight as high as $2.6 \cdot 10^7$ showed some permeation behaviour.
Therefore the porosity of the SI 4000 gel was measured
by titration, according to the method of Mottlau and
Fischer (15). About 1 g of dry gel was weighed in a 50
cm^3 Erlenmeyer flask in which the titrating solvent (ben_
zene) was slowly introduced by means of a plunger micro_
burette; the gel was continously stirred and the flask
was connected to a benzene reservoir in order to elimi_
nate solvent evaporation during the titration. The end
point was taken when a small increment of liquid added
to the gel, forming a very thin layer on the external
surface of the particles, made them stick together to
form a coherent caked mass. The specific porosity $V_{p,sp}$
of SI 4000 could then be calculated, and by multiplying
it with the quantity of packing in the column, the po_
re volume V_p was obtained; from the latter and from the

elution volume of a totally permeating molecule, V_t,
the interstitial volume $V_o = V_t - V_p$ was calculated. The
titration method was checked, for our silica gels, by
measuring the V_p of SI 500 and comparing it with the
value obtained by SEC, with very good agreement (see
Table 2).

RESULTS AND DISCUSSION

Characterization and calibration

From the particle size distributions as determined
at intervals of 2 μm on the micrographs, the number a_
verage and weight average diameters reported in Table
1 were calculated according to the relations given by
Dawkins et al. (16).

The results show small differences in the average
particle size dimensions and narrow size distributions,
particularly for SI 100 and SI 500. A narrow size dis_
tribution of particles is needed for high efficiency
columns (16).

TABLE 1. Number Average and Weight Average Particle
Diameters d_n and d_w and Polydispersity of Si_
lica Particles.

Silica	d_n (μm)	d_w (μm)	d_w/d_n
SI 100	9.90	10.91	1.10
SI 500	8.20	9.30	1.13
SI 1000	8.14	9.67	1.19
SI 4000	7.66	9.10	1.19

The calibration curves, log M vs V_e, of the single columns packed with the different silica gels were determined at an eluent flow rate of about 0.5 cm^3 min^{-1} and are shown in Fig. 1. Toluene was the totally permeating solute. It can be seen from the figure that SI 500 and SI 1000 gels have quite similar pore dimensions, and that the SI 4000 column shows a lower pore volume
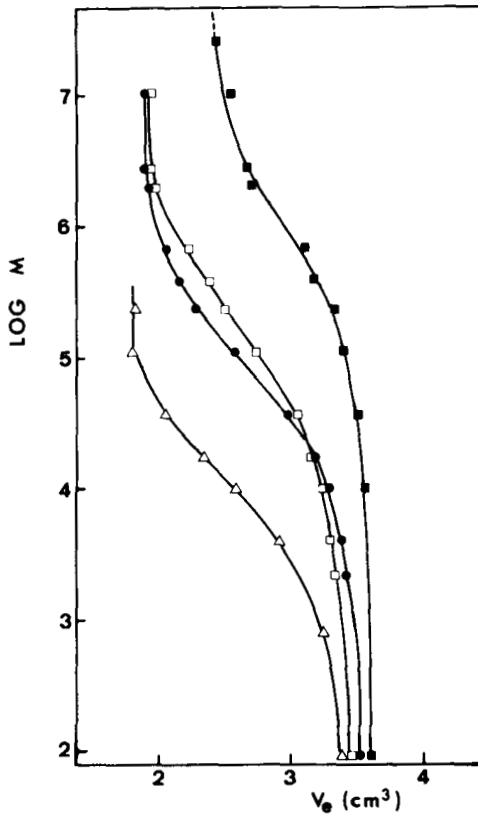

FIGURE 1. SEC calibration curves for polystyrene standards; △, SI 100 silica; ●, SI 500 silica; □, SI 1000 silica; ■, SI 4000 silica.

than the others. From the calibration data the quanti_
ties of interest in SEC can be extracted: V_o and V_t from
the elution volumes of excluded and totally permeating
solutes respectively, V_p from the difference $V_t - V_o$,
and V_s, the volume of solid silica in the column, from
the relation $V_s = V_{col} - (V_o + V_p)$, where the volume of
the empty column, V_{col}, was 4.155 cm^3. With the V_s va_
lues and the measured densities of the solid part of
the microspheres the quantity of silica in the different
packed columns was calculated. (For the SI 4000 column
V_p was obtained from the specific pore volume determi_
ned by titration).

In Table 2 are reported all the characteristics da_
ta of the packed columns. The SI 4000 column has a si_
gnificantly lower quantity of gel than the other columns
and this fact brings about the low value of V_p, together
with the high value of V_o. It appears that the smaller

TABLE 2. Density Values of the Silica Particles and
Characteristics of the Packed Columns.

Silica	Density g cm^{-3}	Grams of Packing	V_o	V_p cm^3	V_t	V_s
SI 100	2.03	1.54	1.79	1.60	3.39	0.76
SI 500	2.11	1.33	1.89	1.64 1.65*	3.53	0.63
SI 1000	2.27	1.57	1.92	1.55	3.47	0.69
SI 4000	2.22	1.24	2.36*	1.24*	3.60	0.56

* by pore titration

dimensions of these particles make it more difficult
to obtain a superior quality packing. For all the co_
lumns the efficiency determined according to eq. (3)
from the toluene peak gave values higher than 20000
plates m^{-1}.

Dependence of H on flow rate

In Fig. 2 are reported the dependences of H on e_
luent flow rate for different solutes eluted in the Li_
Chrospher SI 100 column. Toluene is a totally permea_
ting molecule, PS 10000 and PS 37000 are partially per_
meating PS standards, and PS 111000 and PS 233000 are
two excluded PS standards.

It can be seen that for toluene there is a very
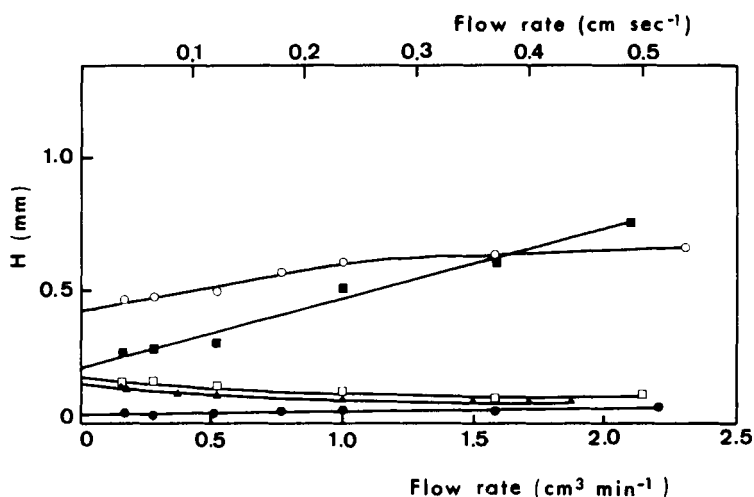low decrease of the H values with decreasing flow rate,

FIGURE 2. Dependence of plate height on flow rate for SI
100 silica: ●, toluene; ○, PS 10000; ■, PS 37000;
□, PS 111000; ▲, PS 233000.

without any tendency of H to increase at the lowest
flow rates investigated. In terms of eq. (9) this means
that the contribution of the longitudinal diffusion in
the mobile phase (term B) can be neglected; the polydi_
sperity term is non operative for toluene, and the small
dependence of H on flow rate indicates that the term
for solute mass transfer in the stationary phase (term
C) is not very important for molecules having high valu_
es of D_s. Therefore the main contribution to H for a
small molecule comes from the eddy diffusion in the mo_
bile phase (term A). Also the excluded polymers show a
low variation of H with flow rate. For these solutes
term C vanishes (1-R = 0), and the polydispersity term
is not to be taken into account because there is no per_
meation into the pores. The slight increase of H at the
lowest flow rates cannot be attributed to term B becau_
se, for the very low values of D_m of these molecules the
longitudinal diffusion is negligible at the flow rates
employed; the effect shown in Fig. 2 is similar to that
observed by Knox and McLennan (8) on solute dispersion
of excluded polymers, and attributed to a partial pene_
tration of the macromolecular coils into the largest
pores of the packing, which becomes evident at low flow
rates. Such an interpretation is in agreement with the
higher values of H for PS 111000 than for PS 233000,
and with the finding of highly tailed chromatographic
peaks at the low flow rates.

For the two permeating polymers term C and the fo_
urth term of eq. (9) are important in determining the

values of H and their dependence on flow rate. The in_
crease of H with increasing flow rates depends on the
ratio $R(1 - R)/D_s$ of term C, whereas the vertical dis_
placement of the curves with respect to the H values of
toluene results from the polydispersity of the samples.
The flattening experimentally found in the curve of PS
10000 at $u > 1$ cm^3 min^{-1} is not accounted by eq. (9); a
decrease of the slope of these curves with increasing
eluent velocities was reported by van Kreveld and van
den Hoed (17) to occur at linear velocities higher than
ours: such an effect increased with increasing molecu _
lar weight of the solute. It has been suggested that at
high flow rates flow into the pores could facilitate
the mass transfer between the mobile and the stationary
phase, but no rigorous interpretation of this phenome_
non is available; a better description of mass transfer
effects within the pores should be supported by a detai_
led knowledge of the pore structure.

The curves showing the dependence of H on eluent
flow rates for the SI 500, SI 1000 and SI 4000 columns
are reported in Figs. 3-5.In these columns excluded po_
lymers, when available, were not analysed at different
flow rates because the very high molecular weight stan_
dards gave broad and distorted peaks which were diffi_
cult to measure. For the other solutes the behaviour
was analogous to that of SI 100. The elution of tolue_
ne at the different flow rates gave practically no va_
riation of H. With SI 4000 the peaks of toluene were
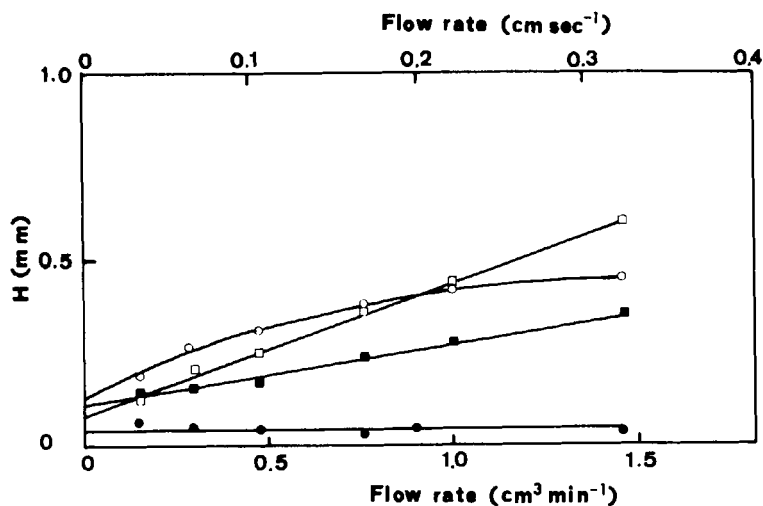somewhat less symmetrical than for the other columns,

FIGURE 3. Dependence of plate height on flow rate for SI
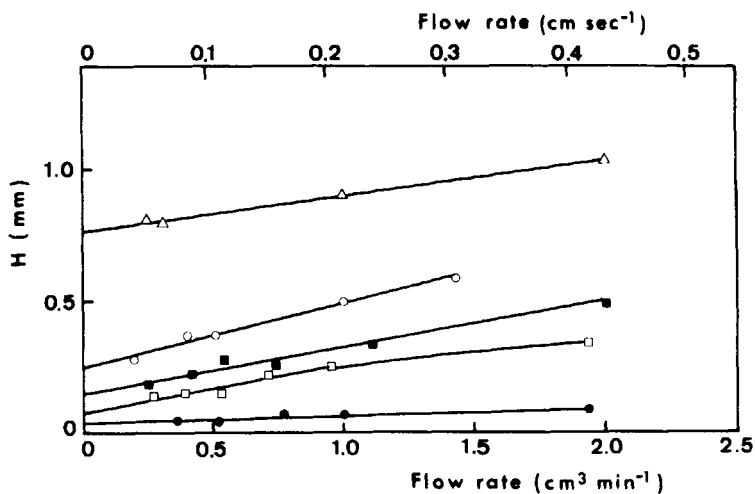500 silica: ●, toluene; ■, PS 37000; □, PS 111000;
○, PS 390000.

FIGURE 4. Dependence of plate height on flow rate for SI
1000 silica: ●, toluene; □, PS 37000; ■, PS
111000; ○, PS 390000; △, PS 2050000.
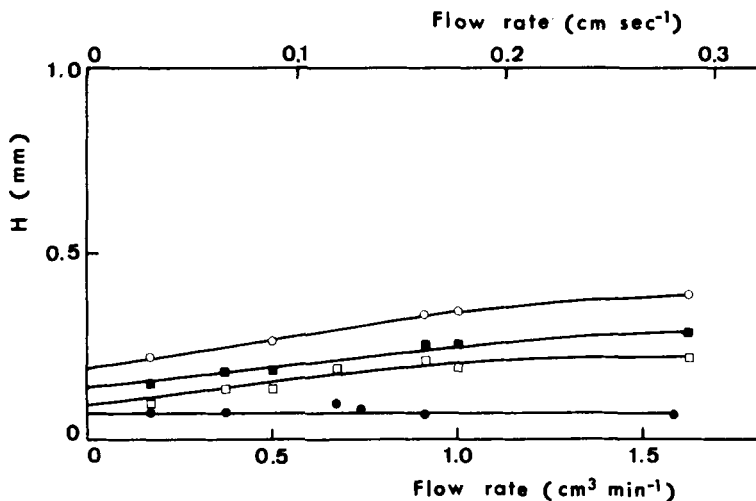
FIGURE 5. Dependence of plate height on flow rate for SI
4000 silica: ●, toluene; □, PS 111000; ■, PS
390000; ○, PS 670000.

and this might be attributed to the less homogeneous
packing bed obtained with this gel. The permeating po_
lymers show higher H values than for toluene, increasing
with flow rate because of the mass transfer of the so_
lute in the stationary phase. The expected dependence
of H on flow rate is also evident in the SI 1000 column
for the elution of PS 2050000, which permeates only a
small portion of pores (see Fig. 1). The strong verti_
cal displacement of the curve for this standard with
respect to the other curves clearly indicates that the
polydispersity of the polymer is not very low.

Diffusion coefficients of polystyrenes in the pores

From the slope of the linear part of the H vs linear
velocity curves of Figs. 2-5 the term of solute disper_

sion resulting from mass transfer of the permeating sam_
ples in the stationary phase can be obtained. When the
packing material is constituted by a collection of sphe_
rical particles with a size distribution, the value of
the configuration factor q is 1/30 (12), and the correct
mean particle diameter $\overline{d}_p$ must be introduced in term C
of eq. (9). The $\overline{d}_p$ which is to be applied is the volu_
me-weighted average value (12), which for particles of
the same material is equal to the weight average diame_
ter d_w. Therefore term C of eq. (9) becomes

$$\frac{R(1 - R)\ d_w^2}{30\ D_s}$$

and the values of the diffusion coefficients D_s of po_
lystyrenes in the pore structure are easily calculated
from the experimental slopes of Figs. 2-5. The D_s va_
lues can be compared with the diffusion coefficients
at infinite dilution, D_m, of the polymers in free solu_
tion, and the ratio D_s/D_m, representing the hindrance
to the molecule diffusion in the pores, is obtained.
The values evaluated for the four silica gels investi_
gated are reported in Table 3. The D_m values for the
PS standards were calculated according to the relation_
ship

$$D_m\ (cm^2\ sec^{-1}) = 3.45 \cdot 10^{-4}\ M_w^{-0.564}$$

valid in tetrahydrofuran at 24 °C for PS samples in the
molecular weight range up to about $2 \cdot 10^6$ (18).

From the data of Table 3 one can see that for the
same molecule eluting in different silica gels the D_s
value increases with increasing mean pore size, as it
is to be expected, but all the D_s values are much lower
than the D_m values, indicating that the diffusion of
the macromolecules in the pores is severely hindered.
The results obtained in this study are completely com_
parable with those reported in the Literature for dif_

TABLE 3. Diffusion Coefficients of Polystyrene Standards
Evaluated on LiChrospher Silicas with Mean Par_
ticle Dimensions d_w.

Standard	Column	d_w (μm)	$D_s \cdot 10^8$	$D_m \cdot 10^8$ ($cm^2 sec^{-1}$)	D_s/D_m
PS 10000	SI 100	10.91	11.23	190.00	0.06
PS 37000			4.24	92.00	0.05
PS 37000			8.84	92.00	0.10
PS 111000	SI 500	9.30	3.52	49.70	0.07
PS 390000			1.81	24.00	0.07
PS 37000			8.89	92.00	0.10
PS 111000	SI 1000	9.67	8.46	49.70	0.17
PS 2050000			2.51	9.70	0.26
PS 111000			12.30	49.70	0.25
PS 390000	SI 4000	9.10	9.97	24.00	0.41
PS 670000			5.84	18.00	0.32

ferent packings: Giddings et al. (12) reported a value $D_s/D_m=0.17$ for low molecular weight polymers in porous glass ($\bar{d}_p=59$ μm); Dawkins and Yeadon (19, 7) have deter_ mined on silica microspheres D_s/D_m values in the range 0.11-0.19 ($\bar{d}_p \approx 20$ μm) and 0.08 - 0.15 ($\bar{d}_p \approx 8$ μm), and similar results were obtained by Knox and McLennan (8), who reported D_s/D_m values in the range 0.06 - 0.17 ($\bar{d}_p \approx$ 7.5 μm), and by van Kreveld and van den Hoed (17) who calculated on Porasil silica ($\bar{d}_p =75 - 125$ μm) values decreasing from 0.312 to 0.119 by increasing the mole_ cular weights of PS standards from 20000 to 160000. The D_s/D_m values of Table 3 vary from 0.05 to 0.41 in the different column systems, and no definite trend is shown by changing the molecular weights in the same si_ lica gel. It can be suggested that the restriction ra_ tio D_s/D_m varies not only with the packing porosity and with the molecular weight of the eluted samples but al_ so with differences in the internal pore structure or in the pore size distribution. Therefore to interpret D_s/D_m variations a more detailed knowledge of the real pore structures would be needed. In any case, it can be concluded that, independently of the particle size dimensions, the transfer of solute molecules from mobi_ le to stationary phase in the pororus particles used in SEC is very slow.

Polydispersities of the polystyrene standards

From the linear part of the curves of permeating polymers in Figs. 2-5 it is possible to extrapolate the intercept value H_{PS}^{int}. By assuming that this value appro_

ximates the plate height H_{PS}^* at very low eluent flow ra_
tes, but where   the term B of eq. (9) would not be
large   and considering that at these low flow rates
the contribution to H of term C is negligible with re_
spect to the eddy diffusion term, one obtains from eq.
(9):

$$H_{PS}^{int} \simeq H_{PS}^* = 2\lambda d_p + (L \ln \frac{M_w}{M_n} / D_2^2 \, v_e^2) \qquad (10)$$

Therefore the plate height H_{PS}^{int} of eq. (10) is the
sum of an eddy diffusion term and a term deriving from
the sample polydispersity. The results shown above in_
dicate that the eddy diffusion term can be well appro_
ximated by the plate height value of toluene H_t^{int} cal_
culated at u = 0; this value can be substituted for
$2\lambda d_p$, and the second term on the right hand side of
eq. (10) can be evaluated. From this term the values
M_w/M_n of the dispersities of the permeating polymers
have been calculated in the different column systems
and the results are shown in Table 4.

The values of M_w/M_n calculated from plate height
data are quite reasonable, according to the characteri_
stics of these "monodisperse" standards; the relative_
ly high dispersity found for PS 2050000 is also in a_
greement with the fractionation data given by the sup_
plier, and with the general higher dispersity of these
high molecular weight standards. The results of Table
4 compare well with those obtained by other Authors on
different PS standards, prepared as well by living ani_
onic polymerization (6 - 8). However, having investiga_

TABLE 4. Polydispersity of Polystyrene Standards Cal_
          culated from Eq. (10).

Polymer	Column	H_{PS}^{int} (mm)	H_t^{int} (mm)	$\bar{M}_w/\bar{M}_n$
PS 10000	SI 100	0.430	0.030	1.073
PS 37000	SI 100	0.210	0.030	1.020
	SI 500	0.110	0.040	1.017
	SI 1000	0.080	0.040	1.019
PS 111000	SI 500	0.088	0.040	1.009
	SI 1000	0.150	0.040	1.042
	SI 4000	0.100	0.070	1.040
PS 390000	SI 500	0.130	0.040	1.043
	SI 4000	0.140	0.070	1.038
PS 670000	SI 4000	0.190	0.070	1.062
PS 2050000	SI 1000	0.770	0.040	1.160

ted the performance of silica particles with different
pore size distributions, the present evaluation of the
polydispersities of PS standards covers a broader mole_
cular weight range. The agreement obtained in the re_
sults for the same polymer in different columns is re_
levant and further supports the applicability of this
chromatographic procedure for evaluating band broadening

in high performance SEC, in spite of the approximations
involved in the calculations.

CONCLUSIONS

The application of classical chromatographic equa_
tions, adequately modified, to high performance SEC has
proved to be a useful tool in the investigation of the
relative importance of the different processes which
determine the band broadening, and in the evaluation
of the permeability characteristics of the porous pac_
kings. In high performance SEC the main dispersion pro_
cess occurring in the mobile phase is the eddy diffusion
of solute molecules. Its contribution to plate height is
minimized by using packings made of small spherical par_
ticles with narrow size distribution. For high molecu_
lar weight polymers, permeating the porous material, the
band broadening is also determined, in addition to the
size fractionation due to the polydispersity of the so_
lute, by the mass transfer of the polymer molecules in
the stationary phase, which is a slow and velocity de_
pendent process. It follows that also at even lower
linear velocities   the plate height contribution from
this term is by no means negligible, at least for poly_
mers having low dispersities. On the other hand, for po_
lymers broadly distributed the contribution from poly_
disperdity will quickly predominate over the dispersion
phenomena occurring in the mobile phase, and with the
values of the mass transfer coefficients generally found
in the SEC packings, it should be possible to obtain re_

liable molecular weight distribution data working at a
bout 0.1 cm sec^{-1} without broadening corrections.

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