HIGH-PERFORMANCE GEL PERMEATION CHROMATOGRAPHY WITH A NEW TYPE OF SILICA PACKING MATERIAL

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SUMMARY

Highly porous silica beads in the 10-μm size range with graduated pore sizes were tested as packings for the separation of polystyrene standards. As a result of the high internal porosity of the packings, relatively large capacity factors of the solutes and selectivity factors up to 4.0 were achieved. On 25-cm columns at $\bar{u} = 0.1$ cm/sec the following numbers of theoretical plates were obtained: for ethylbenzene, 4000; for PS 5, 1000; and for PS 411, 400. For all columns, the plot of log $\bar{M}_w$ *versus* $N^{1/2}$ gave a linear relationship.

The selectivity and column efficiency were demonstrated by separating a mixture of PS 411, PS 98, PS 51, PS 19, PS 5 and PS 2 at a flow-rate of 0.1 ml/min in 45 min on a 25-cm column.

INTRODUCTION

In contrast to other methods of liquid chromatography (LC), separation in gel permeation chromatography (GPC) is largely controlled by the type of packing used in the column, and the choice of an appropriate packing in order to obtain high selectivity and efficiency is therefore the main problem in GPC. In general, a suitable packing should have the following properties:

(a) available as rigid beads, which allow a reproducible and dense packing of the column;

(b) small particles in the 5–10-μm size range, in order to minimize peak broadening in the column;

(c) high porosity of the porous beads in order to obtain a large intra-particle volume for fractionation;

(d) packings with graduated pore sizes, which are suitable for the separation of polymers in a wide molecular-weight range;

(e) chemical inertness of the surface in order to prevent adsorption of the solutes;

(f) sufficient thermal stability to permit chromatography at higher tempera-
tures.

The highest column efficiencies have been achieved with rigid polystyrene[1] and
porous silica packings[2]. However, polystyrene is not available in a small particle-size
range and the porous silicas that are commercially available have a relatively low
porosity[2,3].

Using a new procedure, we have prepared porous silica beads with a high
porosity in different pore-size ranges[4,5]. The beads are stable under high pressures and
can be produced in the 10-μm size range and smaller. By surface reaction, a dense layer
of alkyl groups is chemically bonded on the surface, which allows polystyrenes, poly-
ethylenes and polypropylenes to be fractionated without an adsorption effect[6].

In the present work, the newly developed silica packings have been investi-
gated for the separation of polystyrene standards. Selectivity and column efficiency
are discussed on the basis of the general equations for LC.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was a UFC 1000/05 (Hewlett-Packard,
Frankfurt, G.F.R.) with a specially designed injection head housing a captive septum
(supplied by Prof. I. Halász, Saarbrücken, G.F.R.) and a fixed-wavelength UV detec-
tor (254 nm, 4-μl cell volume) also supplied by Prof. Halász. The columns were 25.0
and 12.5 cm long and 4.0 mm I.D. and 6.0 mm O.D., made of precision-bore stainless-
steel tubing, SS Type 304 (Nennen Chemical GmbH, Dreieichenhain, G.F.R.).
Column blanks were carefully cleaned before use. Porous stainless-steel plugs, Type
PSSH, of 1/10 in. thickness (Pall GmbH, Sprendlingen, G.F.R.) were fitted in the
column outlet. Connections were made with 6-mm Ermeto fittings (Ermeto Arma-
turen GmbH, Bielefeld, G.F.R.).

The columns were packed using the balanced-density slurry technique, as
described by Strubert[7]. Samples were injected with a 5-μl syringe, Type HP 305 (G.
Schmidt, Hamburg, G.F.R.).

Chemicals

The solvent tetrahydrofuran (THF) (E. Merck, Darmstadt, G.F.R.) was care-
fully distilled. Polystyrene standards with a narrow molecular-weight distribution
(Waters Messtechnik, Frankfurt, G.F.R.) were used as samples in the GPC experi-
ments. The concentrations of samples in THF were 0.1 and 0.05 % (w/w). The specifi-
cations for these standards are listed in Table II.

Ethylbenzene was of analytical-reagent grade (Merck). The porous silica beads
were prepared by hydrolytic polycondensation of polyethoxysiloxane[4,5]. Samples were
air-sieved into narrow-sized fractions. The particle size was characterized by the
cumulative undersize distribution in terms of the d_{p50} value. The data for the packings
are given in Table I.

All silica samples were silanized with trimethylchlorosilane, yielding a surface
concentration of 4.5 μmole/m^2 of trimethylsilyl groups[8].

Samples 2, 5 and 6 are identical with Lichrospher Si 100, Si 500 and Si 1000,
respectively.

TABLE I

DATA FOR SILICA PACKINGS

The number of the packing corresponds to the number of the column.

No.	Particle size, d_{p50} (μm)	Porosity	Mean pore diameter, $\bar{D}^*$ (Å)	Exclusion limit for polystyrenes in THF
1	3	0.71	60	$6 \cdot 10^4$
2	10	0.78	100	$1 \cdot 10^5$
3	10	0.82	125	$2 \cdot 10^5$
4	10	0.82	160	$5 \cdot 10^5$
5	10	0.67	500	$5 \cdot 10^5$
6	10	0.62	1000	$\sim 5 \cdot 10^6$

* $\bar{D}$ relates to the highest value in the pore-volume distribution curve.

Calculations

The separation efficiency and selectivity were calculated using the basic equations of LC[9]. Corrections were made only for the dispersity of the polymer samples[10]. As t_0 the retention time of a totally excluded polymer was used.

RESULTS AND DISCUSSION

Selectivity

Selectivity in GPC relates to differences in mean molecular weight and molecular size, and can be expressed in terms of relative retention, α. However, α values obtained in GPC are relatively small compared with those in other methods of LC, because the k' values of solutes are small $(0 < k' < 1.0)$ and do not differ appreciably. The maximum k' value is obtained for the corresponding monomer, whereas for the totally excluded polymer $k' = 0$.

On the basis of the classical equation

$$v_R = v_0 + K_0 v_S$$

we can conclude that only the volume between $v_R = v_0$ $(K_0 = 0)$ and $v_R = v_0 + v_S$ $(K_0 = 1)$ can be used for the separation; v_S corresponds to the intra-particle volume of the column, which is proportional to the total specific pore volume of the porous packing. The intra-particle volume can be expressed in terms of porosity, ε_p, which is related to the total porosity of the column by the equation

$$\varepsilon_t = \varepsilon_0 + \varepsilon_p (1 - \varepsilon_0)$$

where

$$\varepsilon_0 = \frac{v_0}{v_K} = \text{interstitial porosity};$$

$$\varepsilon_P = \frac{v_P}{v_T} = \text{internal porosity};$$

v_0 $\quad$ = interstitial volume of the column;
v_K $\quad$ = volume of the empty column;
v_P $\quad$ = total pore volume of the porous packing;
v_T $\quad$ = total volume of the porous packing.

Using this expression, we obtain for the volume ratio

$$\frac{v_S}{v_0} = \frac{\varepsilon_p\,(1 - \varepsilon_o)}{\varepsilon_o}$$

In order to improve the separation range, v_S must be increased relative to v_0.

The porous silica packings that are commercially available have ε_p values of 0.5–0.7, and with $\varepsilon_0 \approx 0.4$ we obtain volume ratios of 0.75 and 1.05, respectively. The porosity, ε_p, of the samples used in this investigation is much higher (see Table I). With $\varepsilon_p = 0.82$, the volume ratio is calculated to be 1.25. We have also prepared silica packings with ε_p up to 0.9, which seems to be a limiting value with respect to the mechanical stability of the material.

In order to increase the selectivity, we are interested in porous samples in which the high pore volume is distributed in a certain pore-size range. This can be controlled by measuring the pore-volume distribution. We obtained the highest porosities for silica samples in the pore-size range between 60 and 500 Å (columns 1–4). This pore size matches a molecular-weight range between $5 \cdot 10^3$ and $5 \cdot 10^5$.

Samples with larger pores exhibit a smaller porosity (columns 5 and 6). As can be seen in Table II, the k' and α values are relatively high compared with those obtained on columns packed with Porasil[11] or Zorbax[2]. Furthermore, the highest selectivity factors for each column shift to higher mean molecular weights in the sequence from column 1 to column 6, which corresponds to an increase of the mean pore diameter of packings in the same sequence (compare Table I).

Calibration plots for the six columns are given in Fig. 1.

TABLE II

k' AND RELATIVE RETENTIONS (α) OF POLYSTYRENES ON COLUMNS 1–6

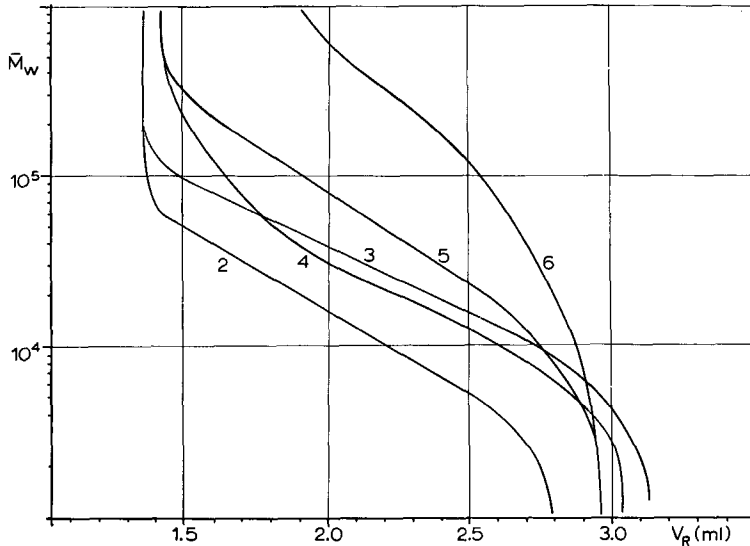| Polystyrene	$\overline{M_w}$	Dispersity, $d=\dfrac{\overline{M_w}}{\overline{M_n}}$	Column 1 k'	Column 1 α	Column 2 k'	Column 2 α	Column 3 k'	Column 3 α	Column 4 k'	Column 4 α	Column 5 k'	Column 5 α	Column 6 k'	Column 6 α
(Ethylbenzene)	106	1.00	1.15		1.20		1.42		1.39		1.09		0.69	
				1.12		1.09		1.05		1.08		1.05		1.04
PS 0.6	600	1.10	1.02		1.11		1.36		1.28		1.04		0.66	
				1.32		1.23		1.13		1.22		1.05		1.06
PS 5	5 000	1.10	0.77		0.90		1.20		1.05		0.99		0.63	
				1.38		1.32		1.19		1.30		1.13		1.03
PS 10	10 300	1.06	0.56		0.68		1.01		0.81		0.88		0.61	
				2.29		1.35		1.23		1.34		1.13		1.08
PS 19	19 850	1.06	0.25		0.51		0.82		0.60		0.78		0.56	
				4.21		4.08		2.05		2.31		1.36		1.17
PS 51	51 000	1.06	0.06		0.12		0.40		0.26		0.57		0.48	
				1.66		3.76		3.36		2.28		1.74		1.19
PS 98	98 200	1.06	0.04		0.03		0.12		0.12		0.33		0.40	
				1.03		1.2		3.53		1.35		1.95		1.22
PS 173	173 000	1.06	0.03		0.03		0.03		0.09		0.17		0.33	
				—		1.5		1.56		5.51		8.89		2.13
PS 411	411 000	1.06	—		0.02		0.02		0.02		0.02		0.16	
				—		—		—		—		—		3.25
PS 867	867 000	1.15	—		—		—		—		—		0.05	
				—		—		—		—		—		5.00
PS 2145	2 145 000	1.20	—		—		—		—		—		0.01	

Fig. 1. Calibration plots for different columns (L = 25.0 cm; I.D. = 4.0 mm). Samples: polystyrene standards. Solvent: THF. Temperature: 25°. The numbers on the curves refer to the column and packing in Table I.

Efficiency

High efficiency in GPC requires the generation of a large number of theoretical plates (N) per column length. This can be realized using columns that are well packed ($\varepsilon_0 \approx 0.4$) with very small particles. However, using small particles the column length is restricted to about 10 cm. In order to increase the total number of theoretical plates, these short columns should be connected without a significant loss in efficiency[3]. In this study, we used 25-cm columns with 10-μm silica particles and a 12.5-cm column with 3-μm particles.

For the monomer ethylbenzene, we obtained the following N values:
column 1 (k' = 1.15):
$\bar{u}$ = 0.1 cm/sec; N = 3100 plates/12.5 cm.
$\bar{u}$ = 1.0 cm/sec; N = 3000 plates/12.5 cm.
column 3 (k' = 1.42):
$\bar{u}$ = 0.1 cm/sec; N = 4400 plates/25 cm.
$\bar{u}$ = 1.0 cm/sec; N = 2400 plates/25 cm.

The N values of the polymers were normalized by dividing the peak width by the polydispersity, d, of the samples (see Table II) according to the equation[10]

$$N = 16 \left[\frac{v_R}{(w/d)} \right]^2$$

The N values of solutes in GPC are largely influenced by the mean molecular weight of the dissolved polymers. From the experimental results, one obtains a linear relationship between the logarithm of $\bar{M}_w$ and the square root of the number of theoretical plates (see Fig. 2). With increasing mean molecular weight, the number of theoretical plates decreases considerably owing to the decrease in the diffusion coefficient, D,
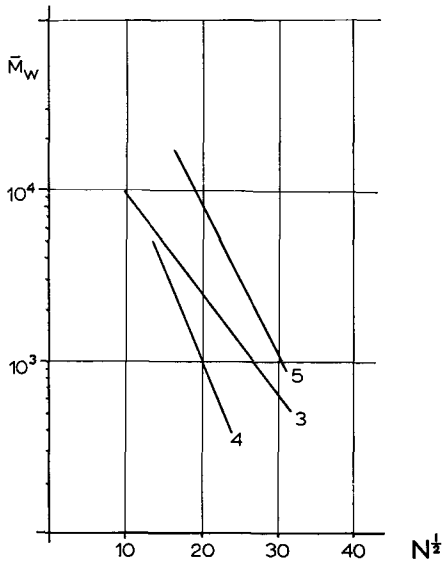
Fig. 2. Log $\overline{M}_w$ versus $N^{\frac{1}{2}}$ for polystyrenes at $\bar{u} = 0.1$ cm/sec. The numbers on the curves refer to columns.

with increasing $\overline{M}_w$. The N values of polymers with $\overline{M}_w > 1 \cdot 10^5$ are in the range 300–400 per 25 cm at $\bar{u} = 0.1$ cm/sec (column 6), whereas the polymers with low molecular weight ($\overline{M} \approx 5 \cdot 10^3$) have N values of about 1000 per 25 cm at $\bar{u} = 0.1$ c/sec (column 3).

A comment should be made about surface modification, e.g., deactivation of the porous silica packing. As shown in earlier experiments[6], hydrophobization of the silica packing with trimethylchlorosilane results in an appreciable decrease (25%) in the plate height (H value) for polystyrenes compared with that of the unmodified products.

Resolution

The resolution, R_s, can be discussed on the basis of the equation

$$R_s = \frac{1}{4} \left(\frac{\alpha - 1}{\alpha} \right) N^{1/2} \left(\frac{k'_2}{1 + k'_2} \right) = \frac{1}{4} \left(\frac{\alpha - 1}{\alpha} \right) N_{eff.}^{1/2}$$

The separation selectivity, as measured by α, is determined by the k' values, while the separation efficiency, as measured by N, is influenced by the particle size, d_p, of the support, the column length, L, and the linear velocity, $\bar{u}$, of the mobile phase. The last term of the equation is proportional to the fraction of the solute in the stagnant mobile phase within the porous particles.

With decreasing α in the range 1.2–1.02, the number of effective plates required for a given resolution increases considerably. As the α values in GPC fall in this range, a high column efficiency is required. However, $N_{eff.}$ is also a function of k'. When k' is initially small ($k' < 1.0$), the value of the function $k'/(1 + k')$ increases sharply with increase in k', in contrast to the value with $k' \gg 1$. This shows the importance of increasing the small k' values by increasing the volume ratio, independently of the

selectivity. The volume ratio, however, is limited by the porosity, ε_p, of the packing. Limiting parameters exist not only for k' but also for N. With a common liquid chromatograph, a maximum pressure of about 4500 p.s.i. can be achieved. On the other hand, the smallest column length, $L_{min.}$, that can be used with very small particles is about 10 cm. Starting with these boundary conditions, we can evaluate R_s as a function of the column pressure, p, separation time, t, and the properties of the column.

If the column length is doubled while p is kept constant, the resolution, R_s, increases by a factor of 1.62 (ref. 9). Doubling the column length, however, results in an increase in the separation time of the same order of magnitude. If the pressure is lowered to half of its initial value while L is kept constant, the separation time will increase by a factor of 2, whereas R_s increases only by a factor of 1.15.

Taking the particle size of the packing into account, a decrease in particle size also leads to an increase in resolution and separation time. A simple calculation[*] shows that when using a 10-cm column with 3-μm particles instead of a 25-cm column with 10-μm particles, while p is kept constant, R_s increases by a factor of 1.5 and the separation time by a factor of 2.2. On the basis of these relationships, we can determine the optimal separation conditions for a given problem.

In practice, we have to compromise between resolution and separation time, as shown in Figs. 3–5. The separation of complex mixtures in GPC requires a high resolution and results in a long separation time. On the other hand, mixtures of a few components that differ widely in their mean molecular weights can be separated quickly.

The analysis time in LC is expressed in terms of effective plates per second which are generated by the chromatographic system. In Table III are listed the $N_{eff.}/t$ values of some samples used in this investigation.
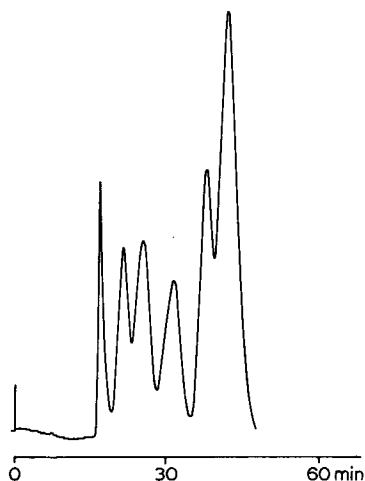

Fig. 3. Chromatogram of a mixture of polystyrenes PS 411, PS 98, PS 51, PS 19, PS 5 and PS 2 on column 3 ($L = 25.0$ cm; I.D. $= 4$ mm). Solvent: THF. Flow-rate: 0.1 ml/min. Pressure drop: 30 p.s.i. Temperature: 25°.

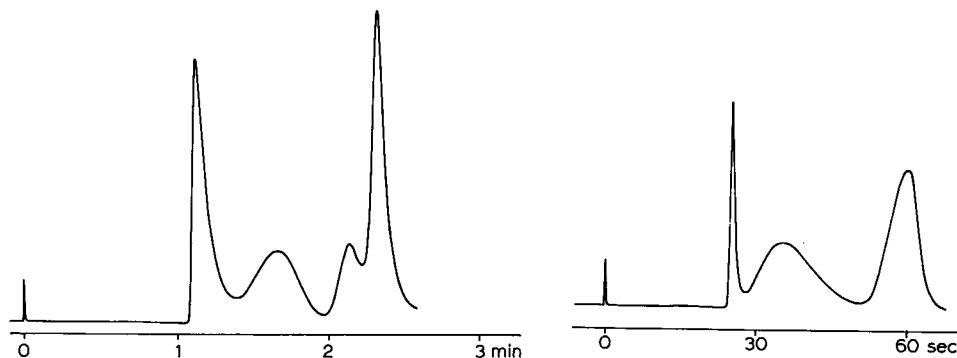[*] Using the empirical equations $H = \text{constant} \cdot d_p^{1.5}$ and $K_0 = \text{constant} \cdot d_p^2$.

Fig. 4. Chromatogram of a mixture of polystyrenes PS 51, PS 10, PS 2 and ethylbenzene on column 1 (L = 12.5 cm; I.D. = 4 mm). Solvent: THF. Temperature: 25°. Flow-rate: 0.5 ml/min. Pressure drop: 470 p.s.i.

Fig. 5. Chromatogram of a mixture of polystyrenes PS 411, PS 51 and PS 2 on column 3 (L = 25.0 cm; I.D. = 4 mm). Solvent: THF. Temperature: 25°. Flow-rate: 3.1 ml/min. Pressure drop: 1100 p.s.i.

From these data, we could conclude that there is an advantage in effecting chromatography at higher linear velocities. This is valid for the monomer, but not for the polymer samples. For the polymers, the $N_{\text{eff.}}/t$ values increase slightly with increasing $\bar{u}$, particular in the region $\bar{u} > 1.0$ cm/sec. Furthermore, in order to maintain diffusional equilibrium in the column, high linear velocities should be avoided.

An additional possibility for decreasing the analysis time in GPC is to chromatograph at higher temperatures. As the viscosity of the solvent depends on the temperature, the linear velocity decreases at constant pressure. This effect is now being studied.

Summarizing the results, we can conclude that highly porous silica packings in the 10-μm size range are suitable for rapid separations in GPC. Complex mixtures can be separated by GPC using highly porous packings in the 3–5-μm size range.

TABLE III

EFFECTIVE PLATES PER SECOND AT DIFFERENT LINEAR VELOCITIES, $\bar{u}$, FOR POLYSTYRENES ON COLUMN 5 (L = 25.0 cm; I.D. = 4 mm)

Polystyrene	k'	$\bar{u}$ (cm/sec)				
		0.05	0.36	1.05	1.51	1.80
(Ethylbenzene)	1.09	0.41	7.46	7.77	17.33	26.91
PS 0.6	1.04	0.40	4.81	6.50	9.10	8.57
PS 5	0.99	0.34	1.24	3.02	4.05	4.28
PS 10	0.88	0.27	1.15	1.56	1.77	2.12
PS 19	0.78	0.16	0.77	1.36	1.49	1.32
PS 51	0.57	0.10	0.33	1.03	0.64	0.68
PS 98	0.33	0.08	0.15	0.3	0.34	0.37
PS 173	0.17	0.02	0.06	0.13	0.12	0.15

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