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COLUMN PARAMETERS CONTROLLING RESOLUTION IN HIGH-PERFORMANCE GEL PERMEATION CHROMATOGRAPHY

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SUMMARY

In gel permeation chromatography, the specific resolution depends primarily on the selectivity, S, and secondly on the column efficiency. S can be improved to a certain extent by utilizing a highly porous packing, as shown by porous silica supports. According to the width of the pore volume distribution of the packings, a broad or a narrow fractionation range can be spanned.

As S is still rather poor, the column efficiency, expressed in terms of the number of theoretical plates (N) per unit column length, should be as high as possible. This can be achieved by adjusting the column parameters and conditions as follows. Well packed columns should be employed with micro-particles in the 5–10-μm size range; the column temperature should be high; the column length may vary between 25.0 and 100.0 cm; and the linear velocity (u) of the eluent may be optimal in the range $0.1 < u < 1.0$ cm/sec. In this way, rapid high-performance separations can be effected.

INTRODUCTION

Although the major developments in gel permeation chromatography (GPC) were made in the mid-1960s[1,2], a renaissance can be now observed, the primary objectives being to improve the resolution and shorten the analysis time. Recently, considerable advances in high-performance liquid chromatography (HPLC) have been achieved by employing highly efficient columns packed with micro-particles[3], and efforts have now been made in the same direction in GPC.

The column performance in GPC is largely controlled by a set of parameters such as packing porosity, pore volume distribution and mean particle size of the packing, column length and temperature and flow-rate of the eluent[2]. As a result, new packing materials have been developed that make it possible to effect rapid high-performance separations[4,5]. The objective of this work was to investigate systematically the influence of the properties of packings and other column parameters on resolution, using tailor-made silica supports and polystyrene standards as solutes.

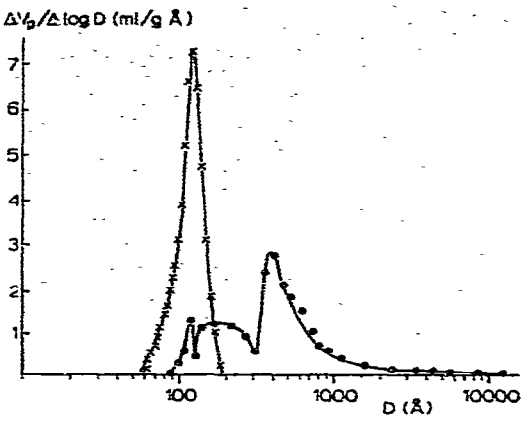

Fig. 1. Differential pore volume distribution of sample 1 (×) obtained by means of nitrogen desorption measurements and sample 4 (●) obtained by means of mercury porosimetry.

EXPERIMENTAL

Packings

Sample 1 was prepared by hydrolytic polycondensation of poly(ethoxysiloxane)[6,7]. Samples 2 and 3 were produced from sample 1 by a controlled sintering at an appropriate temperature[8]. Samples 4–6 were prepared by a slightly modified hydrolytic polycondensation procedure[9,10].

All samples are highly porous. Samples 1–3 show a homogeneous pore volume distribution (PVD), whereas those of samples 4–6 are relatively broad and heterogeneous[10] (see Fig. 1). The pore structure data were obtained by means of nitrogen sorption and mercury penetration measurements. The samples were air-sieved in narrow size fractions of about 10 μm. The properties of the packings are listed in Table I.

TABLE I

PROPERTIES OF THE PACKINGS

The designation of the packings corresponds to that of the columns, *e.g.*, Column 1 is packed with sample 1.

No.	*Mean particle diameter*		*Mean pore diameter*		*Specific surface area,* s_{BET} (m^2/g)	*Porosity,* ε_p (%)
	d_{p50} (μm)*	σ (μm)**	D (Å)***	σ (Å)**		
1	10	3	127	27	300	78
2	11	3	440	125	45	67
3	10	3	1230	470	15	63
4	14	5	70– 600§		363	82
5	17	6	70–1100§		301	86
6	20	5	70–1200§		316	87

* d_p at 50% of the cumulative undersize distribution.
** Standard deviation of the distribution, assuming a gaussian profile.
*** D corresponds to the maximum value of the differential pore volume distribution.
§ Range of D in the pore volume distribution, as PVD is heterogeneous.

Apparatus

A DuPont Model 830 liquid chromatograph was used with a fixed-wavelength ultraviolet (UV) detector (254 nm, 8-μl cell volume). The columns were 25.0 cm long and 4.2 mm I.D., made of precision-bore Type 304 stainless-steel tubing (Nennen Chemicals, Dreieichenhain, G.F.R.). Empty columns were carefully cleaned before use. Porous stainless-steel plugs (Type PSSH) of 1/10-in. thickness (Pall, Sprendlingen, G.F.R.) were fitted in the column outlet. Connections were made with 1/4-in. Swagelok fittings.

The columns were packed by the balanced-density slurry technique[3], and were coupled with low dead-volume capillary tubing in order to minimize band broadening. An additional column, packed with 10-μm particles, was connected to the outlet of the UV detector cell in order to prevent vaporization of the eluent at higher column temperatures.

Samples were injected with a Type HP 305 5μl syringe (G. Schmidt, Hamburg, G.F.R.).

Chemicals

The solvent, tetrahydrofuran (THF) (E. Merck, Darmstadt, G.F.R.), was carefully distilled before use. Polystyrene standards with a narrow molecular weight distribution (Waters Messtechnik, Frankfurt, G.F.R.) were used. The concentrations of samples in THF were 0.1 and 0.05% (w/w). Ethylbenzene of analytical-reagent grade (E. Merck) was chosen as a t_{R0} marker to calculate the linear velocity (u) of the eluent.

RESULTS AND DISCUSSION

Resolution in GPC

Based on the definition of resolution in HPLC[3], Bly[2] derived for GPC the more useful specific resolution parameter, R_S, which is normalized to the mean molecular weight (MW) of the sample and its polydispersity, d:

$$R_S = \frac{2\,[V_{R(2)} - V_{R(1)}]}{(w_1/d_1 + w_2/d_2)\log MW_{(1)}/MW_{(2)}} \tag{1}$$

where

V_R = retention volume of polymer (ml);
w = peak width at the baseline (ml);
d = polydispersity of the polymer, defined as MW_w/MW_n

The subscripts refer to the samples; $V_{R(2)} > V_{R(1)}$.

Eqn. 1 is valid only in the linear range of the calibration graph of log MW *versus* V_R. R_S is independent of the sample and characterizes the ability of the column, under the operating conditions used, to separate two samples that differ in their mean molecular weight.

As known from fundamental equations in HPLC[3], the resolution depends primarily on the column selectivity, S, and secondly on the column efficiency, and the same is true of R_S. A measure of S in eqn. 1 is the ratio k:

$$k = \frac{V_{R(2)} - V_{R(1)}}{\log MW_{(1)}/MW_{(2)}} \tag{2}$$

k is equal to the reciprocal of the slope of the linear logarithmic calibration graph in a certain range. It may be noted that the calibration graph need not necessarily be linear over the whole fractionation range.

The column efficiency is usually expressed in terms of the total number of theoretical plates (N) per unit column length. For GPC, N is normalized to the polydispersity, d, of the sample[2]:

$$N = 16\, d^2 \left(\frac{V_R}{W}\right)^2 \tag{3}$$

By inserting eqn. 3 into eqn. 1 and rearranging, it can easily be seen that R_S is proportional to $N^{\frac{1}{2}}$. In the following sections, we deal with the column parameters that affect selectivity and efficiency in GPC.

Parameters affecting selectivity

A serious disadvantage of GPC compared with other modes of HPLC is the limited peak capacity. According to the basic equation of GPC[2,3],

$$V_R = V_0 + K_{GPC}\, V_I \tag{4}$$

samples will be eluted only between $V_0 < V_R < V_0 + V_I$, where V_0 is the interstitial volume of the column, V_I corresponds to the total pore volume of the column packing and K_{GPC} is the so-called "distribution coefficient", which varies in the range $0 \leqslant K_{GPC} \leqslant 1$, assuming that only steric exclusion takes place.

Most of the commercially available GPC packings, both organic gels and inorganic materials, exhibit a phase ratio $V_I/V_0 \leqslant 1$, *i.e.*, V_I is in the same order of magnitude as V_0. In order to obtain a large peak capacity in GPC, V_I has to be increased to as large a value as possible relative to V_0. However, certain limitations arise, which will be discussed briefly.

The interstitial volume, V_0, can be expressed in terms of the interstitial porosity, ε_0, which is defined as

$$\varepsilon_0 = \frac{V_0}{V_K} \tag{5}$$

where V_K is the volume of the empty column. ε_0 can be diminished only to about 0.4, which corresponds to the porosity of the densest packing of spheres of equal size[11]. In practice, ε_0 values vary in the range $0.35 < \varepsilon_0 < 0.45$. For a well packed column, ε_0 should be constant at different column temperatures and pressures.

V_I can be also expressed in terms of a dimensionless parameter. The internal porosity, ε_p^K, of the column is defined as

$$\varepsilon_p^K = \frac{V_I}{V_K} \tag{6}$$

ε_p^K is related to the particle porosity, ε_p, by the equation

$$\varepsilon_p^K = \varepsilon_p (1 - \varepsilon_0) \tag{7}$$

and ε_p is calculated from

$$\varepsilon_p = \frac{V_g}{V_g + V_g} \quad (6)$$

where V_g is the specific pore volume of the porous packing (ml/g) and V_g is the specific volume of the purely solid packing (ml/g) or the reciprocal of the density of the packing. Obviously, the particle porosity can be increased to a certain upper limit, which will be determined by the mechanical stability of the particles towards pressure. Using the well known balanced-density slurry technique[5], the particles have to withstand pressures of up to 350 bars. We found that 10-μm silica beads with a maximum ε_p of 0.87 ($V_p = 3.0$ ml/g) can still be packed by means of this technique without any appreciable fractionation. This shows that V_I/V_0 can be increased up to about 1.3 ($\varepsilon_0 = 0.4$). A plot of the phase ratio *versus* the specific pore volume, V_p, of the packing reveals (see Fig. 2) that the optimal value of V_p, with respect to maximal V_I/V_0, is at about 2.0 ml/g. Higher V_p values contribute only a negligibly small amount to the phase ratio. On column No. 4 packed with silica with $V_p = 2.0$ ml/g, a phase ratio of 1.5 could be obtained (see Fig. 3).

A high specific pore volume of the packing distributed in a certain pore size range results in a high selectivity. In a previous investigation[7], it was found possible to prepare highly porous silica beads exhibiting a narrow pore volume distribution in the mesopore size range [30 Å $< D$ (mean pore diameter) $<$ 500 Å]. Such packings (see sample 1) are needed in order to perform separations in a narrow molecular weight range. Silica of larger pore size, such as samples 2 and 3, are made by a controlled sintering of sample 1 (ref. 8). As a result of the high-temperature treatment, the original pores are markedly enlarged, whereas the specific pore volume decreases slightly. These packings match a molecular weight range of up to $2 \cdot 10^6$. A systematic study is now being carried out in order to prepare highly porous silica samples with only

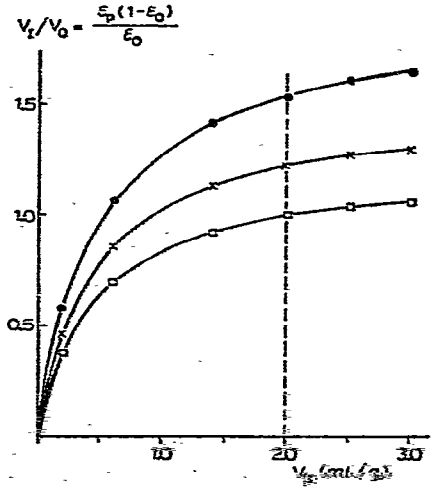

Fig. 2. Column phase ratio, V_I/V_0, expressed in terms of $\varepsilon_p(1 - \varepsilon_0)/\varepsilon_0$ as a function of the specific pore volume, V_g, of the packing. ε_0: ●, 0.35; ×, 0.40; □, 0.45.

micropores or micro- and mesopores to fractionate oligomers (molecular weight < 5000).

In general, we are interested in separating polymers in a broad molecular-weight range, covering several orders of magnitude. Such separations will usually be achieved with a set of columns, coupled in order of decreasing exclusion limits. However, we found that a broad fractionation range can be also spanned by one column, packed with a tailor-made silica packing, instead of using connected columns containing different types of silica.

In contrast to samples 1–3, the newly developed silica types 4–6 are also highly porous but possess a heterogeneous and broad pore volume distribution[9,10]. When carrying out separations, one column packed with sample 4 replaces a column set of samples 1 and 2, and a column packed with sample 5 replaces a set of samples 1, 2 and 3. This result is surprising, because preferably size separations should be performed with packings of a narrow PVD. In our opinion, extreme caution should be exercised in interpreting the PVD of a porous packing and its relationship with the experimental calibration graph, for the following reasons.

Calculations of PVD are based on nitrogen sorption and mercury penetration measurements. Sorption methods cover a pore size range only up to a mean pore diameter, D, of 400 Å (refs. 13 and 14), whereas mercury porosimetry spans a pore size range from 15 μm down to 40 Å (high-pressure mode)[13,15,16]. To cover the whole pore size range, both methods have to be applied. This would require that porosimetry and sorption give the same results with respect to PVD. This cannot be expected, however, because the two methods are based on different assumptions. In particular, porosimetry data must be considered with caution, because the pore walls may collapse under high-pressure treatment[15].

Further, the pore size, *e.g.*, the mean pore diameter, D, is based on the assumption of a simple pore model, except in the modelless case, in which D is expressed as a hydraulic diameter, D_h, corresponding to the ratio of volume to surface area[12–14]. The pore models used are a rather poor approximation of the real pore shape. It must be also emphasized that the measurements are made under static conditions, which differ substantially from those used in GPC. The data obtained represent only an average pore volume distribution and give no information on how the pores are distributed within the porous particle. For instance, the larger pores may be accumulated on the outer surface, whereas the smaller pores are mainly in the inner part, or *vice versa*. The pore arrangement with respect to the size and the shape of the pores mainly determines the size exclusion mechanism. As simple derivations cannot be made from the measured PVD, we assume that samples 4–6 possess an optimal pore arrangement in order to achieve size separations over a broad molecular-weight range. In a study to be published later, we also found that the size exclusion mechanism on these columns and packings is accompanied by restricted diffusion. This effect is particularly pronounced for high-molecular-weight samples and at high flow-rates[10].

Parameters affecting efficiency

In order to achieve a given resolution, a maximum number of theoretical plates (N) should be attained, as an improvement in selectivity is limited by the packing properties. N is equal to the column length (L) divided by the plate height (H):

$$N = \frac{L}{H} \tag{9}$$

It is known that the size of H is influenced by numerous parameters, but here we shall deal only with the dependence of H on the molecular weight of the sample, the linear velocity (u) of the eluent, the mean particle size (d_p) of the packing and the column temperature (T_K).

In GPC, it is more realistic to evaluate N as a function of MW instead of choosing the low-molecular-weight monomer as a standard. The data in Table III confirm that N decreases markedly with increasing MW of the sample at constant T_K and operating conditions. As already indicated by Bly[2], the square root of N is a linear function of log MW of the sample.

Experimental results in the range $0.1 < u < 1.0$ cm/sec reveal that to a first approximation H increases linearly with u for all samples when other conditions (d_p, L and T_K) are held constant. Table II shows that the slope of the $H = f(u)$ graph in the range measured increases with increasing molecular weight of the sample. This effect is due to the diminution of the effective diffusion coefficient, D_{eff}, which decreases with increasing molecular weight of the sample. The data in Table II also suggest that H/u for a given polystyrene is reduced drastically by increasing T_K. Thus, for a high efficiency, u should be small and T_K should be increased as much as possible.

The dependence of H on d_p of the packing in HPLC is well known[3,17,18]. H has been found to be approximately equal to $d_p^{1.3}$ at $u = 1.0$ cm/sec. At lower linear velocities, the exponent of d_p also decreases[19]. Consequently, in order to provide a substantial improvement in H, d_p should be reduced. However, Halász *et al.*[18] have indicated that a series of problems arise when d_p becomes smaller than 5 μm. Thus, with respect to efficiency and speed of analysis, size fractions between 5 and 10 μm should be optimal for liquid chromatographic separations.

In only a few papers has the influence of T_K on resolution in GPC been considered[20,21]. Using cross-linked polystyrene gels as packings, an increase in T_K may change the internal distances in the swollen gel network. As V_K remains constant, V_0 should be diminished and also V_I could be affected, depending on the degree of cross-linking. Cantow *et al.*[20] observed a pronounced decrease in V_R with increasing T_K

TABLE II

SLOPE OF THE LINEAR RANGE OF THE $H = f(u)$ CURVE FOR POLYSTYRENE STANDARDS AT THREE COLUMN TEMPERATURES

Column dimensions: $L = 25.0$ cm; I.D. = 4 mm. Packing: No. 4, $d_{p50} = 10\,\mu$m. Eluent: tetrahydrofuran. Detector: UV (254 nm).

MW_w (polystyrene standards)	H/u (msec)		
	297 °K	323 °K	373 °K
98 000	699	663	523
51 000	618	492	256
19 000	314	245	124
5 000	80	52	21
600	27	13	5
106 (ethylbenzene)	11	4	1.4

TABLE III

N VALUES FOR POLYSTYRENE STANDARDS ON COLUMN 4 AT $u = 0.1$ cm/sec AND DIFFERENT TEMPERATURES

Conditions as in Table II.

MW_w (polystyrene standards)	*N*			
	297 °K	*323 °K*	*373 °K*	*417 °K*
411 000 (excluded)	2601	2315	2100	2427
173 000	130	141	230	235
98 000	151	212	235	298
51 000	220	242	329	329
19 000	363	389	460	465
5 000	1126	1163	1214	1238
600	3125	3571	3677	3968
106 (ethylbenzene)	6757	6803	6898	6945

using polystyrene as standards and 1,2,4-trichlorobenzene as solvent, which is in contrast to the theoretical predictions.

In our work, we established that the V_R values of polystyrenes are independent of the variation in T_K from 293 to 413 °K. This also means that the selectivity remains constant.

On the other hand, an increase in T_K at constant u results in an increase in N (see Table III); N is found to be approximately proportional to T_K. The factor increases with increasing molecular weight in the given fractionation range. According to

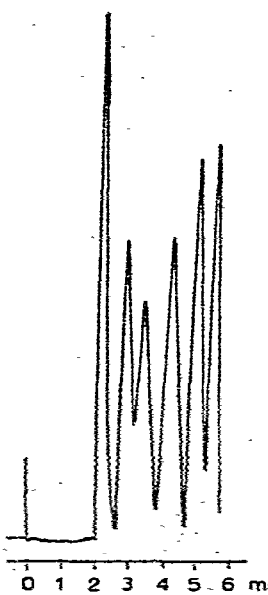

Fig. 3. Separation of a mixture of polystyrene standards. Column, $L = 25.0$ cm, I.D. = 4 mm; packing, sample 4, $d_p = 14$ μm; column temperature, 413 °K; solvent, tetrahydrofuran; injection volume, 5 μl; detector, UV (254 nm); flow-rate, 0.70 ml/min; pressure drop, 100 p.s.i.; elution order, polystyrene *MW* 411 000, 98 000, 51 000, 19 000, 5 000, ethylbenzene.

the Wilke–Chang equation, the diffusion coefficient, D, in the bulk solution is proportional to the temperature[22]. D also depends inversely on the viscosity, η, which decreases slightly with temperature. Strictly, we have to deal with D_{eff} instead of D, D_{eff} being smaller than D, but a quantitative treatment cannot be given because it is difficult to calculate D_{eff}[10]. Another aspect may be the change in the hydrodynamic volume, V_h, of the polymer. As tetrahydrofuran is a good solvent for polystyrene, V_h should hardly change with increasing T_K[23].

To a first approximation, we can assume that the improvement in N and H is due to the increase in D_{eff} of the sample with increasing T_K. It must be emphasized that an increase in T_K at constant u simultaneously reduces the pressure drop, Δp, in the column, because Δp is proportional to the viscosity of the eluent[3]. For instance, at the conditions specified in Tables II and III, at $u = 0.1$ cm/sec Δp decreases by a factor of 2 when increasing T_K from 297 to 373°K. This leads to a reduction in the analysis time of the same order of magnitude. Assuming that analysis time is proportional to t_{R0}, the retention time of the monomer, we obtain

$$\Delta p = \frac{\eta L u}{K} \tag{10}$$

where K is the permeability of the column, and with

$$u = \frac{L}{t_{R0}}$$

$$\Delta p = \frac{\eta L^2}{K t_{R0}} \tag{11}$$

In this way, the analysis time can easily be calculated and optimized for a given separation problem. As shown in Fig. 3, rapid high-performance separations can be achieved within a few minutes on a 25-cm column. If necessary, such columns can be coupled in sets of total length 50, 75 and 100 cm. We found that with coupled columns, the decrease in H can be kept below 10%. With such rapid separations, however some problems arise particularly when a molecular-weight distribution has to be calculated[24].

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