

Monosized polymer particles in size-exclusion chromatography

I. Toluene as solvent

LARS-INGE KULIN and PER FLODIN*

Department of Polymer Technology, Chalmers University of Technology, S-412 96 Gothenburg (Sweden)
and

TURID ELLINGSEN and JOHN UGELSTAD

SINTEF, University of Trondheim, Trondheim (Norway)

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ABSTRACT

Monosized macroporous poly(styrene-divinylbenzene) particles were prepared by a multi-step swelling process. The preparation of particles of different sizes (5, 10 and 20 μm) with similar pore-size distributions allowed a selective evaluation of the effect of particle size on column efficiency. All three particle sizes yielded columns with similar calibration graphs, which was to be expected because of their nearly identical pore-size distributions. The uniform packing which may be achieved with monosized particles resulted in columns with high efficiency and separation capacity. With 5- μm particles more than 50 000 theoretical plates were obtained in a 30-cm column (HETP \approx 0.006 mm). The resolution, as measured by the parameter R_{sp} , was good throughout the experiments and especially high for the 5- μm particles. The theoretical prediction that HETP is proportional to the square of the particle diameter was confirmed by the experimental data.

INTRODUCTION

It is well known that flow-rate and particle size in addition to packing methodology are important for column efficiency. For a certain column the main rule is that smaller particles give higher efficiency, but at the same time the resistance to flow increases, resulting in a higher pressure drop. For various applications suitable compromises between these factors must be found.

The first gel particles designed for size-exclusion chromatography (SEC) were made by cross-linking of the polysaccharide dextran (Sephadex)^{1,2}. Later, gels for the separation of higher molecular weight substances were produced from agar³.

The introduction of gel particles that swell in organic solvents⁴ and later me-

chanically stable, porous particles of poly(styrene-divinylbenzene) (SDVB)⁵ offered the possibility of characterizing polymers in organic solvents. The appearance of much smaller particles of the same type provided a substantial increase in column efficiency, which allowed shorter times for an experiment⁶.

As peak broadening due to mobile phase dispersion is reduced in columns packed with spherical particles having a narrow size distribution⁷, it is obvious that totally monosized particles would be the best choice. Such particles could previously only be approached by repeated mechanical screening. Using the principle of activated multi-step swelling developed by Ugelstad and co-workers⁸⁻¹², it is possible to prepare highly monosized polymer particles with predetermined diameters in the range 1-100 μm . Ion-exchange materials prepared by this method have proved to be extremely efficient in protein separation processes⁹.

This paper reports SEC using monosized, porous poly(styrene-divinylbenzene) particles. The separation efficiency and the flow properties were investigated using narrow polystyrene standards and *o*-dichlorobenzene. The monodispersity of the dry particles was examined by scanning electron microscopy (SEM) and their porous structure by mercury porosimetry and the BET method.

EXPERIMENTAL

Monosized particles

The chromatographic gel materials designed for this investigation were monosized porous SDVB particles⁸⁻¹⁰, made by a two-step microsuspension process, whereby radical bulk polymerizations take place in separate micrometre-sized droplets. In the first step, a low-molecular-weight, water-insoluble compound was introduced into highly monosized 1.0- μm polystyrene seed particles. In the second step the monomer mixture was absorbed in the 'activated' seed particles and polymerized. By introduction of a non-polymerizable diluent in the monomer mixture, the particles were given a macroporous structure.

The polymer particles described were prepared with 50% DVB and with heptane as diluent in an amount of 50 vol.-% of the organic phase. After polymerization, the porogenic agent was removed by repeated washing with isopropanol followed by Soxhlet extraction with methanol. Three batches of particles were prepared with diameters of 5, 10 and 20 μm .

Specific surface areas of the particles were determined by the BET method. Pore volumes and pore radius distributions were measured by mercury porosimetry using a Carlo Erba Model 1500 instrument. A Jeol JSM 840 scanning electron microscope was used to examine the bead structure and the monodispersity of the beads.

Column packing

The gel particles were dispersed and swollen in toluene and traces of fines were removed by decanting. Steel tubes (30 cm \times 7.8 mm I.D.) with 2- μm frits at the inlets and the outlets were used as columns.

Packing was performed in the following way: the column was partially filled with toluene and connected to an extension tube, then the column and the extension tube were filled with a particle slurry. After connecting a pump to the top of the extension tube, solvent was forced through the system at *ca.* 1.0 ml/min until the packing was finished. The pressure increased throughout the packing procedure.

SEC

Standard Waters Assoc. 150C SEC equipment was used for the SEC measurements. Three pulse dampers were located just after the pump in the flow system. Otherwise the equipment was in the original mode with a refractive index detector. Toluene was used as the solvent and the whole system was held at room temperature. The flow-rate was 1.0 ml/min throughout.

Calibration was performed with Waters Assoc. narrow polystyrene standards covering molecular mass ranges from $2.7 \cdot 10^6$ down to 450 g/mol. All standards were dissolved in toluene 2 h before injection. No filtration was needed. The injection volumes varied between 5 and 25 μ l.

Calculations

Using efficient columns producing Gaussian peaks, the "tangent method"¹³ was used to study the band broadening phenomena. The equation used was $N = 16(V_R/w)^2$, where N is the number of theoretical plates and V_R is the retention volume of the peak. The tangents to the inflection points of the elution curve were drawn from the peak to the baseline, where the difference w of the intersections was measured.

The height equivalent to a theoretical plate (HETP) was calculated as L/N , where L is the length of the column. For the calculation of N , the retention volume, V_R , and baseline peak width, w , were measured as positions on the recorder paper and then recalculated as volume. The totally monodisperse peak used was that of the solvent *o*-dichlorobenzene (ODCB).

The resolution parameter, R_{sp} , was calculated according to the definition^{6,14} $R_{sp} = 0.58/\sigma D_2$, where D_2 is the slope of the calibration graph and σ is the peak standard deviation. In the calculations the average σ values of the polystyrene standards within the linear part of the calibration graphs were used. D_2 was also taken from this part of the calibration graph.

RESULTS

Monosized particles

Scanning electron micrographs of the particles demonstrating their high degree of monodispersity are shown in Fig. 1. The BET surface areas and the pore volumes are given in Table I. As shown in Fig. 2, the pore-size distributions are nearly identical for the three particle sizes. The particles contain a wide range of pore sizes, with a maximum at 1800 Å. The separation of the curves at the upper levels is due to the inter-particle void volume, which is directly related to the particle size. The steepness of the curves illustrates the high degree of monodispersity of these materials.

It should be kept in mind that BET, SEM and mercury porosimetry are methods that require drying of the gel materials. Changes in pore sizes by swelling of the porous particles in solvents such as toluene and tetrahydrofuran must be taken into consideration when the data obtained are applied to SEC. The volume swelling of the porous materials M-05A (5 μ m), M-10A (10 μ m) and M-20A (20 μ m) in toluene are 15%, 9%, and 6%, respectively. The experimental data in Table I are for the gel materials in the dried state.

A scanning electron micrograph of the surface structure of the porous particles M-05A is shown in Fig. 3.

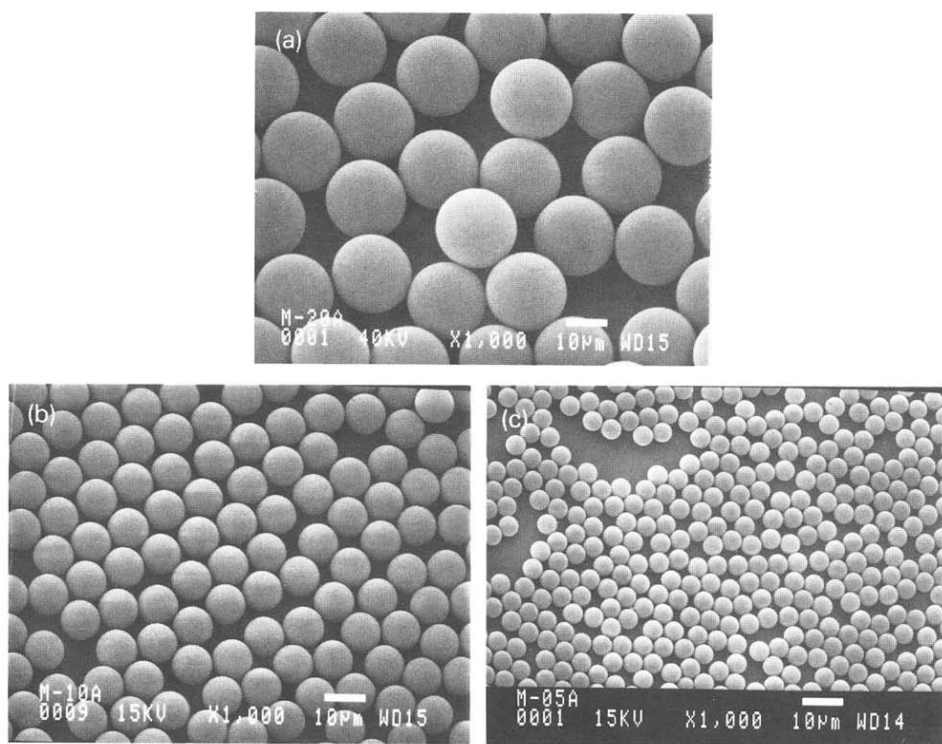


Fig. 1. Scanning electron micrographs of SDVB particles (a) 20 μm (M-20A); (b) 10 μm (M-10A); (c) 5 μm (M-05A).

Size-exclusion chromatography

For each particle size, three or more columns were packed and the performance of each column was examined. Columns producing skewed peaks were rejected, leaving three 20- μm , three 10- μm and two 5- μm columns left to characterize.

Particle diameter 20 μm . In Table II, experimental results obtained with a column packed with 20- μm particles are shown. The reproducibility of the retention volumes of both polystyrene standards and ODCB was good. The N value of ODCB was 3000 ± 200 whereas lower values were obtained for the polystyrenes. The void

TABLE I
CHARACTERISTICS OF GEL MATERIALS

Gel material	Particle diameter (μm)	S_g (BET) (m^2/g)	Pore volume (ml/g)
M-20A	20.6	289	1.12
M-10A	10.5	355	1.12
M-05A	5.25	261	1.04

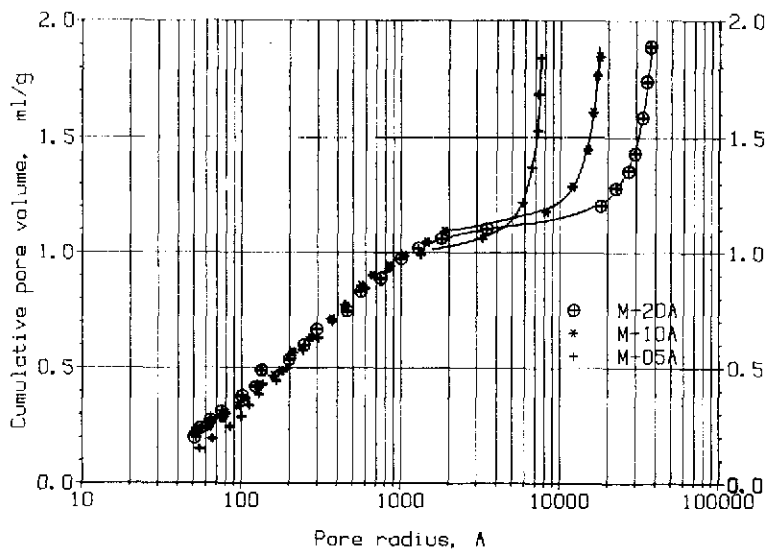


Fig. 2. Cumulative pore volume for 20-, 10- and 5- μ m monosized porous particles. Copolymers with 50% porosity and 50% DVB.

volume was 6.5 ml, which corresponds to 47% of the total volume of the column. The pressure drop was 5 bar at a flow-rate of 1.0 ml/min.

The polystyrene calibration graph, showing separation in the molecular weight range $6 \cdot 10^5$ –450 g/mole, is shown in Fig. 4. The graph is linear between $3 \cdot 10^5$ and $2 \cdot 10^4$ g/mol. The resolution parameter, R_{sp} , was calculated to be 2.4.

For some of the 20- μ m columns, variations in the SEC conditions were applied. It was found that lower flow-rates, smaller injection volumes and higher temperatures gave higher N and R_{sp} values.

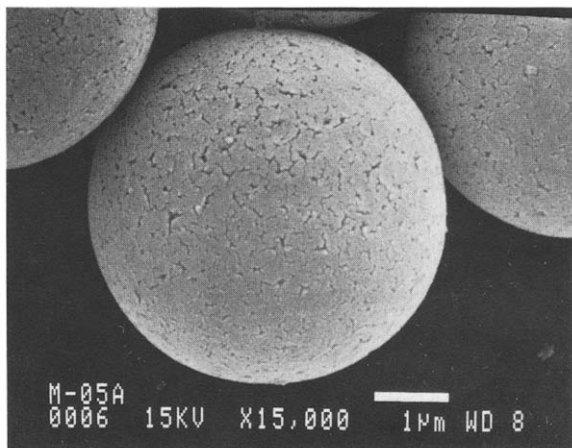


Fig. 3. Surface structure of the porous particles M-05A.

TABLE II
PERFORMANCES OF 20-, 10- AND 5- μm COLUMNS

Parameter	Particle diameter (μm)		
	20	10	5
Void volume (ml)	6.5	8.0	4.7
Pressure drop (bar)	5	8	65
Linear separation range (g/mol)	$3 \cdot 10^5 - 2 \cdot 10^4$	$3 \cdot 10^5 - 2 \cdot 10^4$	$3 \cdot 10^5 - 2 \cdot 10^4$
N	3000	13 000	> 50 000
HETP (mm)	0.1	0.023	< 0.006
R_{sp}	2.4	2.8	4.4

Particle diameter 10 μm . The average results are presented in Table II. An N value of $13\,000 \pm 3000$ was found, which corresponds to a HETP of about 0.023 mm. The calibration graph resembles that of the 20- μm particles with a linear part between $3 \cdot 10^5$ and $2 \cdot 10^4$ g/mol. An average R_{sp} value of 2.8 was found. The pressure drop was 8 bar at a flow-rate of 1.0 ml/min.

Particle diameter 5 μm . The two columns yielded N values of 66 000 and 33 000. Taking $N = 50\,000$ as an average value, the HETP becomes 0.006 mm (Table II). An average R_{sp} value of 4.4 was obtained. From the elution curve in Fig. 5, it is observed

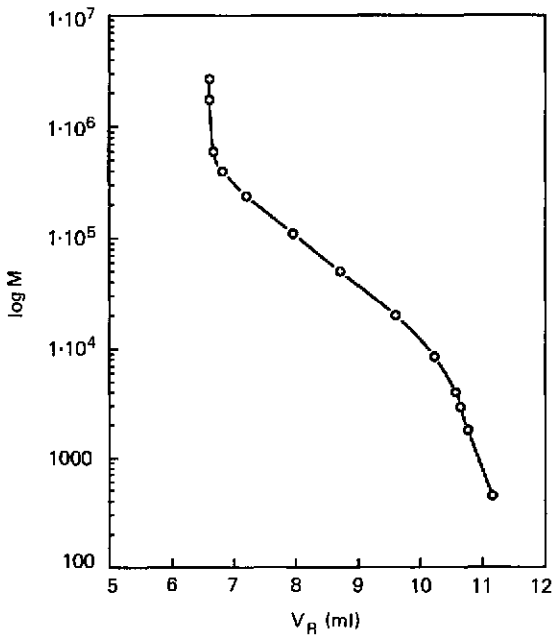


Fig. 4. PS calibration, 20- μm column.

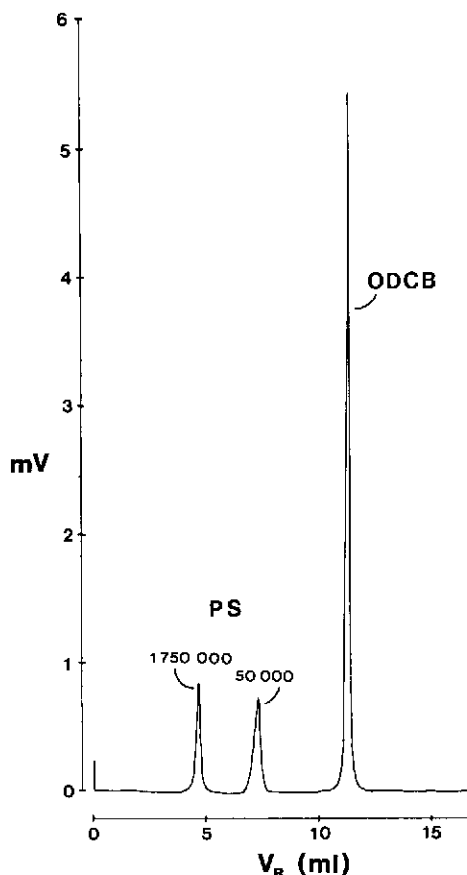


Fig. 5. Example of chromatogram obtained with PS standards ($MW = 1.7 \cdot 10^6$ and $5 \cdot 10^4$) and ODCB, $5\text{-}\mu\text{m}$ column.

that the peaks of the PS standards became narrower the higher the molecular weight. The calibration graph was very similar to that for the other particle sizes. The pressure drop was as high as 65 ± 5 bar and it remained at this level throughout all the experiments. The average void volume was 4.7 ml or 35% of the total volume.

DISCUSSION

With a column I.D. of 7.8 mm and a particle material height of 290 mm, the total particle volume in a column is 13.9 ml. It is well known that a close packing of equally sized spheres occupies 74.0% of the total volume. Accordingly, the void volume of a totally close-packed column will be 3.6 ml. In practice, such a low void volume is not obtained. More realistic void volumes will be 35–40% of the total volume, *i.e.*, in the present instance 4.5–5.5 ml for a well packed column.

It should be noted that the $5\text{-}\mu\text{m}$ particle columns had a small void volume of 4.7 ml when compared with those of the columns packed with larger particles. The

TABLE III
PARTICLE DIAMETER *VERSUS* HETP

d_p (μm)	d_p^2	HETP (μm)
20.6	424	100
10.5	110	23
5.25	27.5	6

high pressure drop of the 5- μm columns could be expected to cause compression of the particles. In such a case an extra and unwanted mobile phase volume would be visible at the top of the packed bed, causing dilution of the samples and band broadening. However, no broadening behaviour was observed, and no permanent compression of the total gel volume was observed when the columns were dismantled.

All three particle sizes yielded columns with similar calibration graphs, as expected owing to the nearly identical pore-size distributions (Fig. 2).

The band broadening decreased substantially with smaller particles. It also decreased, but to a smaller extent, when the flow-rate was decreased or when the temperature was increased. With 5- μm particles more than 50 000 theoretical plates were obtained, corresponding to an HETP of the same order of magnitude as the particle diameter.

For the PS standards, band broadening increased with increasing molecular weight for the columns packed with 20- and 10- μm particles. For those packed with 5- μm particles, however, the bands visually became narrower with increasing molecular weight. An explanation is that the larger the molecules the more excluded they become, and thus the distances to diffuse in and out of the particles decrease. This tends to compensate for the slower diffusion due to higher molecular weights.

The separation power as measured by the resolution parameter, R_{sp} , increased on going from 20- to 10- μm particles. The 5- μm columns gave much higher R_{sp} values and would thus allow the separation of species with closer molecular weights. The advantage of using R_{sp} when characterizing a column is obvious. We recommend that both R_{sp} and N are calculated. N measures the efficiency of the column with respect to particle size, elution rate and packing. To characterize a column with respect to separation efficiency, calculation of the R_{sp} value is needed.

It has been deduced theoretically that the HETP should be proportional to the square of the particle diameter⁶. Monodisperse particles would be ideal for testing this hypothesis. In Table III it is shown that our experimental data are in accordance with the theory. These results give $\text{HETP} \propto d_p^{2.06}$. After correction for swelling in toluene, the exponent becomes 2.09. This is in good agreement with ref. 7.

In a future study, the pore-size distribution will be varied as a parameter. Monosized porous particles are ideal for this purpose, being the only test material that ensures identical bead properties.

CONCLUSIONS

Monosized porous poly(styrene-divinylbenzene) particles can be packed into

columns which are highly efficient for separation of mixtures of PS standards in toluene. The column performance improved considerably with decreasing particle size.

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