High performance g.p.c. with crosslinked polystyrene gels: influence of particle size distribution

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Spherical crosslinked polystyrene gel particles have been separated by air classification into fractions having average particle diameters in the range 10–40 μm. The particle size distributions have been shown to be narrow by Coulter Counter measurements. G.p.c. column performance improves as the average particle diameter decreases and columns packed with gel fractions having a number-average particle diameter below 20 μm give plate counts in excess of 3000 plates per foot. Plate height results as a function of eluent flow rate suggest that chromatogram broadening due to mobile phase dispersion is reduced in columns packed with spherical particles having a narrow size distribution.

INTRODUCTION

Despite the widespread use of crosslinked polystyrene gels for polymer fractionation by gel permeation chromatography (g.p.c.), little work has been reported on the chromatographic performance of gel particles having well-defined particle size distributions. Moore[1] in the first report on the separation of high polymers with semi-rigid macroporous gels described two particle size ranges, 200–325 mesh (50–75 μm) and 100–200 mesh (75–150μm), but he did not compare column efficiencies. Somewhat later, Altgelt and Moore[2] stated that gel particles in the size range 10–100 μm are suitable and that narrower fractions within this range are preferred. A thorough investigation of gel preparation and column packing procedure was performed by Peaker and Tweedale[3]. They obtained plate counts in the range 700–1300 plates per foot with gel particles, in the size ranges 15–63 μm and 15–90 μm. These particles were produced by wet sieving to remove large particles and by sedimentation to remove fine particles. Further studies by Peaker and Tweedale[4] showed that particles in the size range 15–45 μm gave the best column efficiency (916 plates per foot) which decreased as the size range, 45–63 μm, 63–90 μm and 90–150 μm, increased.

The crosslinked polystyrene gels developed by Moore[1] are marketed as Styragel, trade-name of Waters Associates Incorporated. Waters *et al.*[5,6] examined three Styragel columns, having particle size ranges: 10–15 μm, 25–37 μm and 37–42 μm, for high speed g.p.c. but the dependence of column efficiency on particle size was not clearly identified. The development of microparticulate inorganic packings for high performance liquid chromatography[7] suggested that small particles should be suitable for fast g.p.c. separations without loss in resolution. Vivilecchia and coworkers[8] reported a large increase in column efficiency as particle size decreased for the particle size ranges: 37–75 μm (Styragel), 18–25 μm, 8–12 μm and 3–8 μm. This work led to the development of μStyragel, trade name of Waters Associates Incorporated, for high speed g.p.c. determinations of the molecular weight distributions of high polymers[8–10]. Results for columns of μStyragel[8] and for TSK–GEL (trade name of Toyo Soda Manufacturing Co., Ltd)[11,12] with average particle diameters of 20, 10 and 5 μm suggest that the plate counts are in the range 3000–12 000 plates per foot.

In this paper, the g.p.c. performance of crosslinked polystyrene gels with well-defined particle size distributions is examined. Although it has been recognized qualitatively that the particle size distribution will influence the performance of columns packed with microparticulate polystyrene gels[8,9], no quantitative studies have been published previously.

EXPERIMENTAL

Gel preparation

Suspension polymerizations were performed in a wide-necked round-bottomed flask (2 dm^3), baffled with four deep vertical creases and a large dimple on the base impressed into the glass. The flask was fitted with a condenser, thermometer, nitrogen inlet, separating funnel and Citenco constant speed stirrer. The stirring head was a four-blade pitched blade turbine, designed so that its diameter was half of the flask diameter at its widest point. The blades were set at 45° and were positioned 1 in above the top of the dimple. Heating was provided by a constant temperature water bath. The polymerization recipe was: styrene (10 cm^3), divinylbenzene (50 cm^3), benzene (40 cm^3), azobisisobutyronitrile (1.5 g), and a suspension stabilizer (20 g) in water (1 dm^3). The stabilizer was a partly hydrolysed poly(vinyl acetate) ('Gelvatol' 20–60 with a degree of polymerization of 1750 and 80 wt % hydrolysis). The aqueous Gelvatol 20–60 solution at 60°C was purged with nitrogen in the flask. The organic phase (monomers, diluent, initiator) was added and allowed to equilibrate before starting the stirrer at about 600 r.p.m. Polymerizations were performed at 60°C for 14 h. The polymerization product was separated by filtration in

Table 1 Average particle diameters and plate counts of g.p.c. packings

Gel	s_n (µm)	s_w (µm)	s_w/s_n	Plate count (per ft)
Unclassified	19.74	33.89	1.72	–
>28 µm	26.34	35.36	1.34	1110
24–28 µm	27.24	30.60	1.12	1580
20–24 µm	22.41	25.42	1.13	2340
16–20 µm	19.31	23.07	1.19	3390
10–15 µm	13.57	16.17	1.19	4980
ARgeL 805	32.38	36.76	1.14	843

order to remove very fine particles (<1 µm). Batches of gel were mixed, washed three times with water to remove dissolved Gelvatol 20–60, then washed twice with a water: ethanol mixture (50:50) to dissolve the Gelvatol from the surface, and washed with ethanol to remove diluents. The gel was dried in a vacuum oven at 80°C.

Air classification

The dry gel was separated into narrow size ranges with an Alpine Laboratory Zig-Zag Centrifugal Classifier in the Department of Chemical Engineering. The separation is based on the movement of particles under the action of various forces in an air stream, giving a fine fraction removed by the air flow and a coarse fraction[13]. Repetitive separations produced the size ranges given in *Table 1*.

Particle size distribution

A Coulter Counter Model T in the Department of Chemical Engineering was employed for the particle size range 0–80 µm, counting particles in 4 µm intervals. Gel particles were wetted with aqueous Teepol and then dispersed in a large volume of distilled water with an ultrasonic probe. The data were collected automatically and the computer output produced a number fraction size distribution, a weight fraction size distribution, and a cumulative weight size distribution.

Column packing

The apparatus was similar to that described by Peaker and Tweedale[3]. Their balancing and pumping techniques were followed except for the use of toluene as pumping fluid instead of tetrahydrofuran. The pumping rate was gradually increased until the pressure gauge registered 1200 lbf/in^2. Stainless steel columns (0.76 cm internal diameter) were used with end fittings kindly supplied by Applied Research Laboratories Limited (Luton, Bedfordshire). Columns were generally 30 cm in length L, but some shorter (⩾25 cm) and longer (⩽61 cm) columns were investigated with no significant change in plate count (plates per foot).

Gel permeation chromatography

Calibration and plate count determinations (toluene) were performed at 1 cm^3/min with a Perkin–Elmer Model 1220 positive displacement syringe pump (flow settings 0.05–6.00 cm^3/min, ⩽3000 p.s.i., 500 cm^3 capacity) and with an Applied Research Laboratories Limited ultra-violet detector (254 nm, cell volume = 8 µdm^3). The solutes were toluene (Analar) and polystyrene standards (Waters Associates), having a concentration of 0.6% (w/v), and tetraphenylethylene (Aldrich Chemical Co.), having a concentration of 0.02% (w/v). Solutions (10 µdm^3) were injected through a septum arrangement, see *Figure 1*. The syringe needle was pushed through the porous Teflon disc (C) so that the needle touched the centre of the stainless steel mesh (E). The short length of narrow bore tubing (G) connected to the column inlet by the nut (H) minimized dead volume. The eluent was tetrahydrofuran (BDH Chemicals Ltd.) which was destabilized and distilled from calcium hydride before use. The plate height H was calculated from the retention volume V_R and the width of a chromatogram, as described elsewhere[14].

RESULTS AND DISCUSSION

Particle size distribution

The optical micrographs in *Figure 2* confirm the success of the air classification procedure. These gel particles are compared with particles of ARgeL, grade 805, produced by wet-sieving so that particles passed through a 63 µm sieve but were retained on a 37 µm sieve. Particle size distributions are shown in *Figure 3* and were constructed by drawing a curve through the weight fractions at the midpoints of the 4 µm intervals. Average particle diameters were calculated from the number fraction distribution with the relations:

$$s_n = \frac{\Sigma n_i D_i}{\Sigma n_i} \qquad (1)$$

$$s_w = \frac{\Sigma n_i D_i^4}{\Sigma n_i D_i^3} \qquad (2)$$

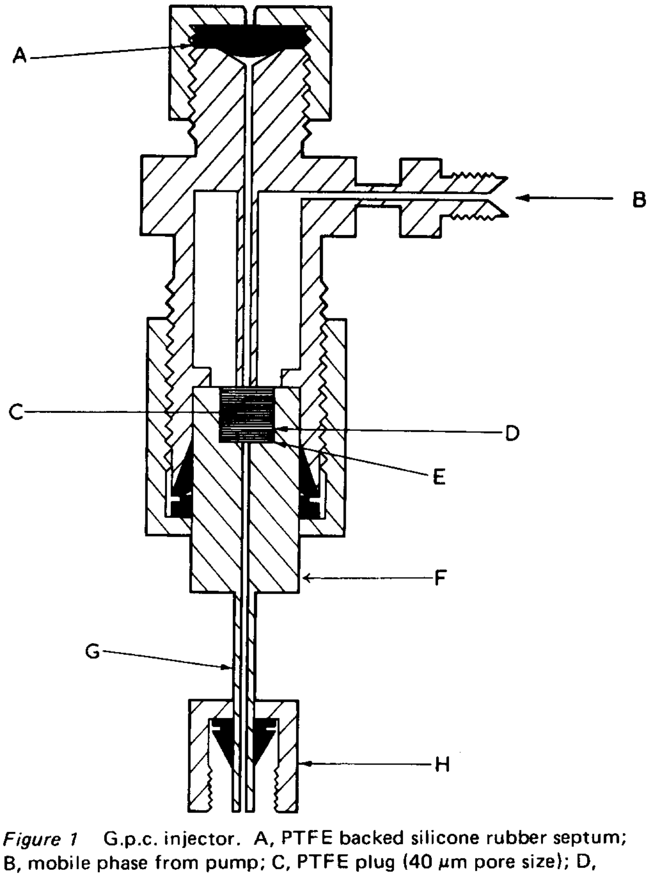

Figure 1 G.p.c. injector. A, PTFE backed silicone rubber septum; B, mobile phase from pump; C, PTFE plug (40 µm pore size); D, 3 X 3mm pocket; E, woven s/s mesh (5 µm pore size); F, 18 X 6.35 mm o.d. s/s tubing; G, 15 X 1.6 mm o.d. s/s tubing; H, nut for 1.6 mm o.d. tubing

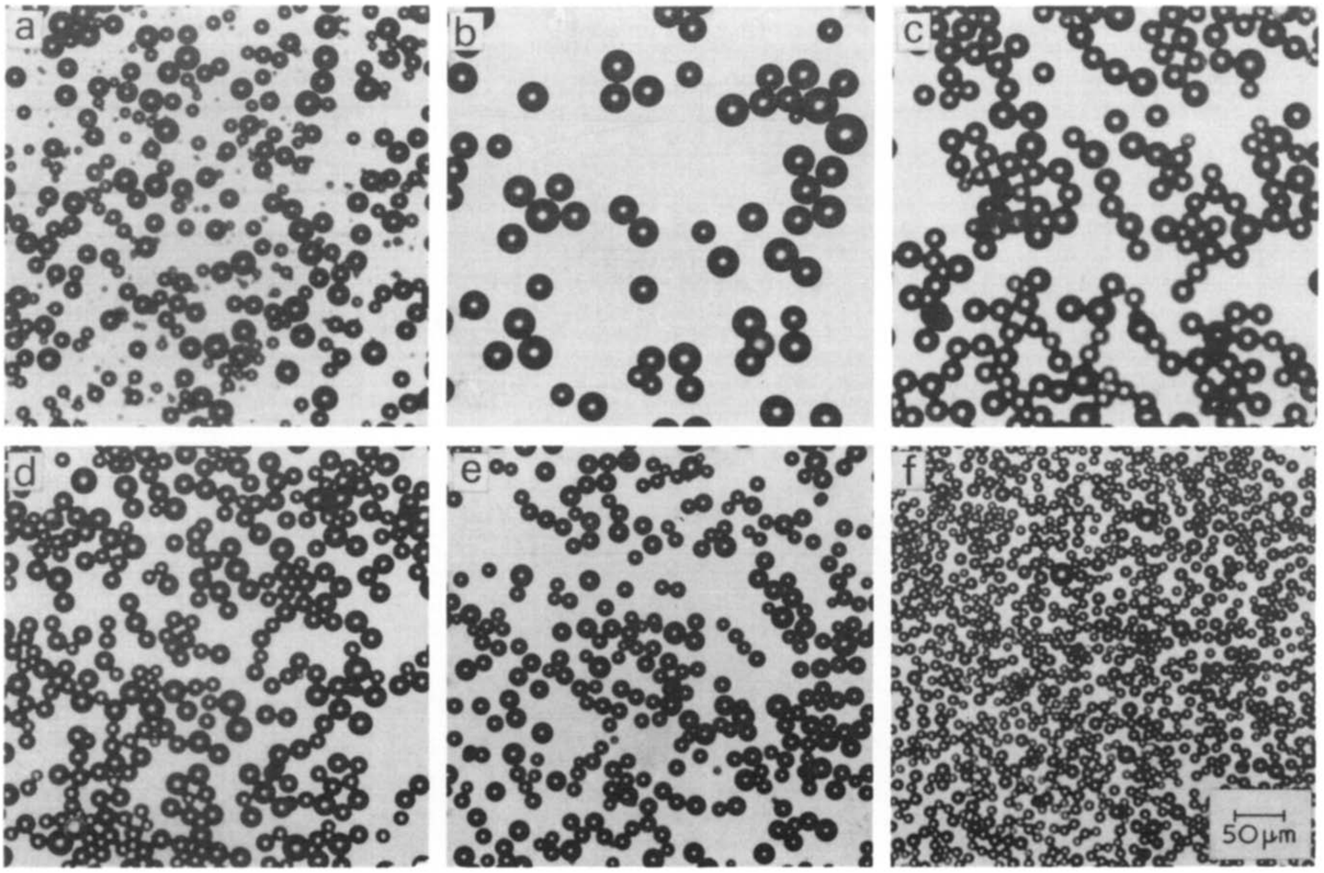

Figure 2 Optical micrographs of gel particles. (a) unclassified; (b) >28 μm; (c) 24–28 μm; (d) 20–24 μm; (e) 16–20 μm; (f) 10–15 μm

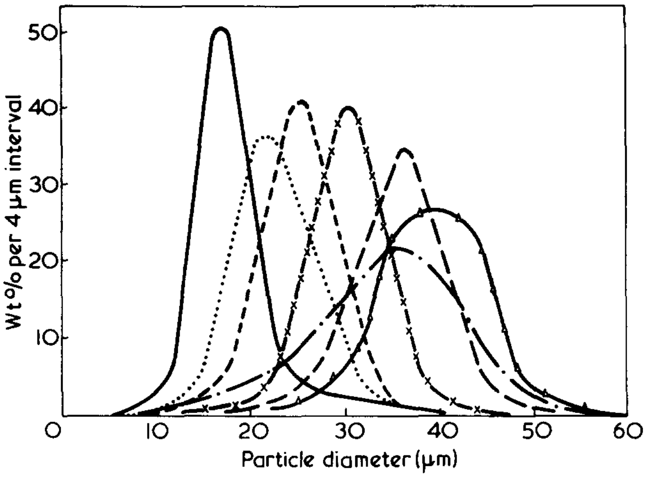

Figure 3 Weight fraction particle size distributions: —·—, unclassified; Δ, ARgeL 805; — —, >28 μm; X, 24–28 μm; — — —, 20–24 μm; · · · ·, 16–20 μm; ———, 10–15 μm

where s_n and s_w are the number-average and weight-average diameters and n_i is the number of particles with diameter D_i corresponding to the mid-point of interval *i*. Values of s_n, s_w and the polydispersity index s_w/s_n for the distributions in *Figure 3* are given in *Table 1*.

The air classifier size range settings in *Table 1* were estimated from calibration curves for particles with a density of 2.5 g/cm^3, by assuming a density of 1.0 g/cm^3 for a crosslinked polystyrene gel particle. Values of s_n fall in these ranges, apart from the >28 μm fraction which was not reclassified in order to decrease the tail at low particle sizes. Values of s_w are close to the peak of the weight fraction size distribution in *Figure 3*. These distributions show that a substantial weight of a gel fraction is outside the 4 or 5 μm size range of the air classifier, so that there is considerable overlap among the fractions as in the fractionation of polymers by the dependence of solubility on molecular weight.

Limpert, Cotter and Dark[9] have suggested that high efficiency columns packed with particles having an average size below 20 μm result when $s_w/s_n < 1.1$ The data in *Table 1* suggest that the upper limit is at least 1.20. One possible reason for the discrepancy may be the calculation of s_w, since Limpert *et al.*[9] state that finding the ratio of s_w/s_n is the same as obtaining a polydispersity number of a polymer. If the expression:

$$s = \frac{\Sigma n_i D_i^2}{\Sigma n_i D_i} \qquad (3)$$

is used incorrectly to calculate s_w, then the ratio s_w/s_n must be lower than when equations (1) and (2) are used. The justification of equation (2) is that the weight of a particle is proportional to D_i^3, and, therefore, $\Sigma n_i D_i^3$ is proportional to the total weight of all the particles.

Gel permeation chromatography

Calibration curves established for columns packed with the gel fractions were very similar (see *Figure 4*) suggesting that the air classification procedure did not segregate particles with different porosities. The calibration curve is similar to curves for 60 and 100 Å Styragel (10 Å = 1 nm) and for ARgeL, grade 805. Plate count values in *Table 1* show the anticipated increase as particle size falls. The plate count

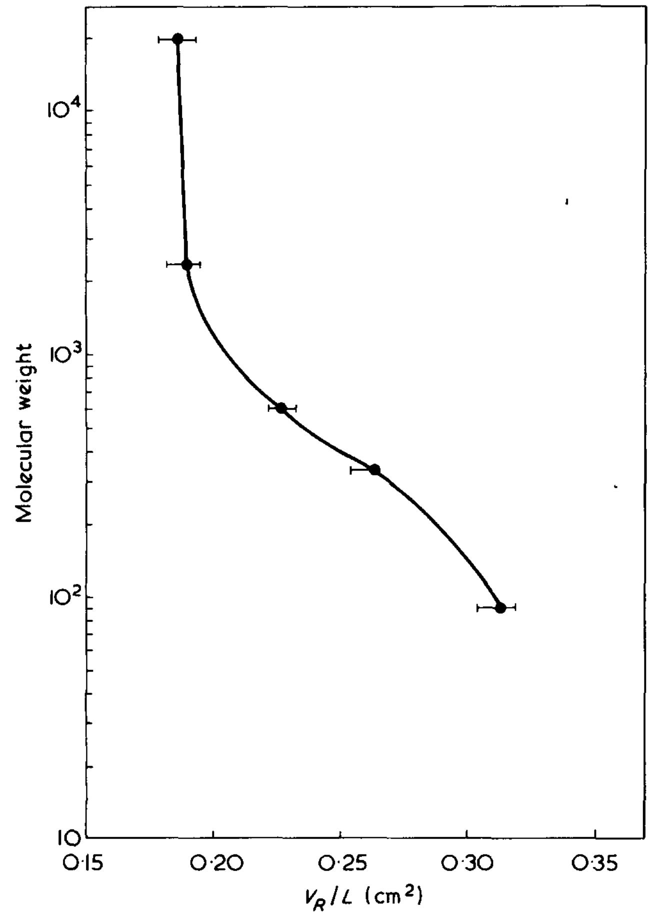

Figure 4 G.p.c. calibration curves for polystyrene standards, tetraphenylethylene and toluene. ●, 10–15 μm gel column; the bars indicate the spread of retention volumes for other columns

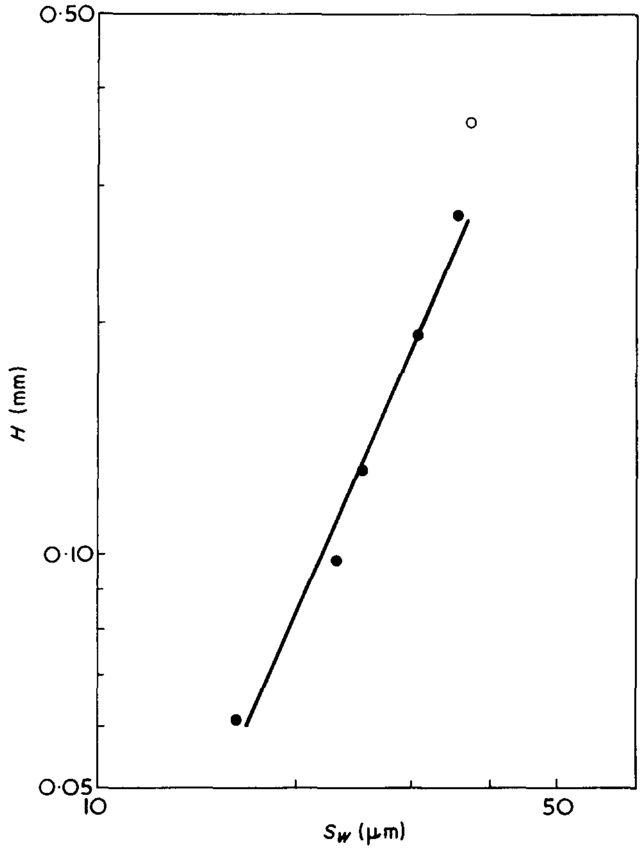

Figure 5 Dependence of plate height on particle diameter

for the 10–15 μm column exceeds the guaranteed minimum plate count of 12 000 plates/m stipulated by Waters Associates for a 100 Å μStyragel column. This result is for particles with a higher average diameter than the value of 10 μm quoted for commercial samples of μStyragel[8–10].

A plot of the plate height *H*, *versus* the weight-average particle diameter is shown in *Figure 5* in which the straight line is the best least squares fit through the filled symbols. The point for the column of ARgeL 805 which was sized by sieving was omitted because of the different shaped distribution, see *Figure 3*.

The straight line obeys the relation:

$$H \propto s_w{}^{1.95} \qquad (4)$$

The exponent may be compared with the value of 1.8 determined by Majors[15] in liquid chromatography separations with silica gels having mean particle diameters in the range 5–40 μm. However, our data in *Figure 5* do suggest that the dependence of H on s_w may be a curve, and a decrease in the exponent as s_w decreases has been observed by several workers in liquid chromatography, see a recent review[16]. If the data are extrapolated as a curve, then H for s_w = 10 μm is about 0.033 mm, giving a plate count of about 9000 plates per foot.

The dependence of column efficiency on eluent flow rate was investigated with a column packed with the 16–20 μm particle size range fraction. Results for plate height are given in *Figure 6* for a non-permeating solute, a polystyrene standard having a molecular weight of 470 000, for a partly per-

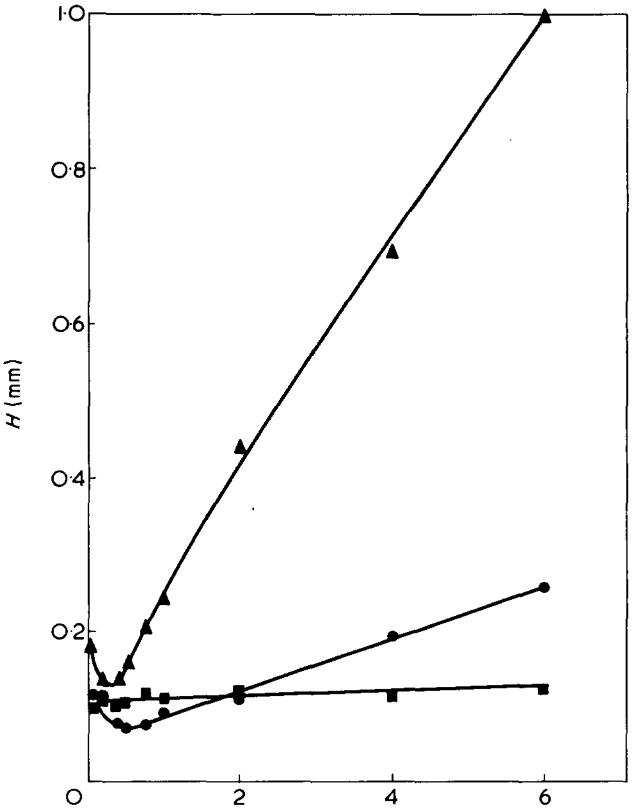

Figure 6 Dependence of plate height on eluent flow rate for 16–20 μm gel. ■, polystyrene standard (470 000); ▲, tetraphenylethylene; ●, toluene

meating solute, tetraphenylethylene (molecular weight = 332), and a completely permeating solute, toluene (molecular weight = 92). The permeation characteristics of these solutes are evident in *Figure 4.* The data in *Figure 6* suggest that optimum column performance is obtained over a restricted range of flow rates. At low eluent flow rates (<0.1 cm^3/min), *H* rises because of solute dispersion due to longitudinal molecular diffusion. At higher eluent flow rates (>0.5 cm^3/min), *H* rises because of the dependence of solute mass transfer between the mobile and stationary phases on flow rate. The divergence of the curves for the permeating solutes as flow rate rises suggests a possible dependence of permeation dispersion on solute diffusivity, as proposed by Kelley and Billmeyer[17,18] for inorganic packings. The data in *Figure 6* suggest therefore that the best column performance is obtained at 0.5 cm^3/min when the plate count is 4225 plates per foot.

Kelley and Billmeyer[17–19] have suggested that a major contribution to *H* for Styragel columns arises from a non-uniform flow velocity profile across a column. They showed that *H* for a non-permeating solute having a low diffusivity hardly changed as a function of flow rate for columns of Styragel and glass particles[17–20]. Our results for polystyrene in *Figure 6* show little variation with flow rate. However, Billmeyer and Kelley[19] interpreted the much higher *H* values for a non-permeating solute having a low diffusivity compared with the values for a permeating solute as evidence for a velocity profile effect. At flow rates below 1 cm^3/min in *Figure 6,* the *H* values for toluene and polystyrene are similar, suggesting that the velocity profile effect is reduced considerably.

A non-uniform flow velocity profile across a column is expected with particles having a wide size range. Particle segregation may occur during column packing which may produce variations in packing density. Variable resistance to fluid flow across a column will then result. Our particles are regular and have a narrow size distribution. Further evidence is presented in *Figure 7,* suggesting that our columns should contain a homogeneous bed of particles. Consequently, mobile phase dispersion due to the velocity profile effect and to eddy diffusion should be reduced with a narrow particle size distribution. We have found that plate heights of about 0.08 and 0.1 mm for toluene and polystyrene are obtained for narrow columns of Spherisorb silica having a mean pore diameter of 8 nm and a mean particle diameter of 20 μm[21]. These narrow columns were slurry-packed and plate heights were obtained at a linear eluent flow velocity identical to the linear velocity corresponding to a flow rate of 1 cm^3/min in

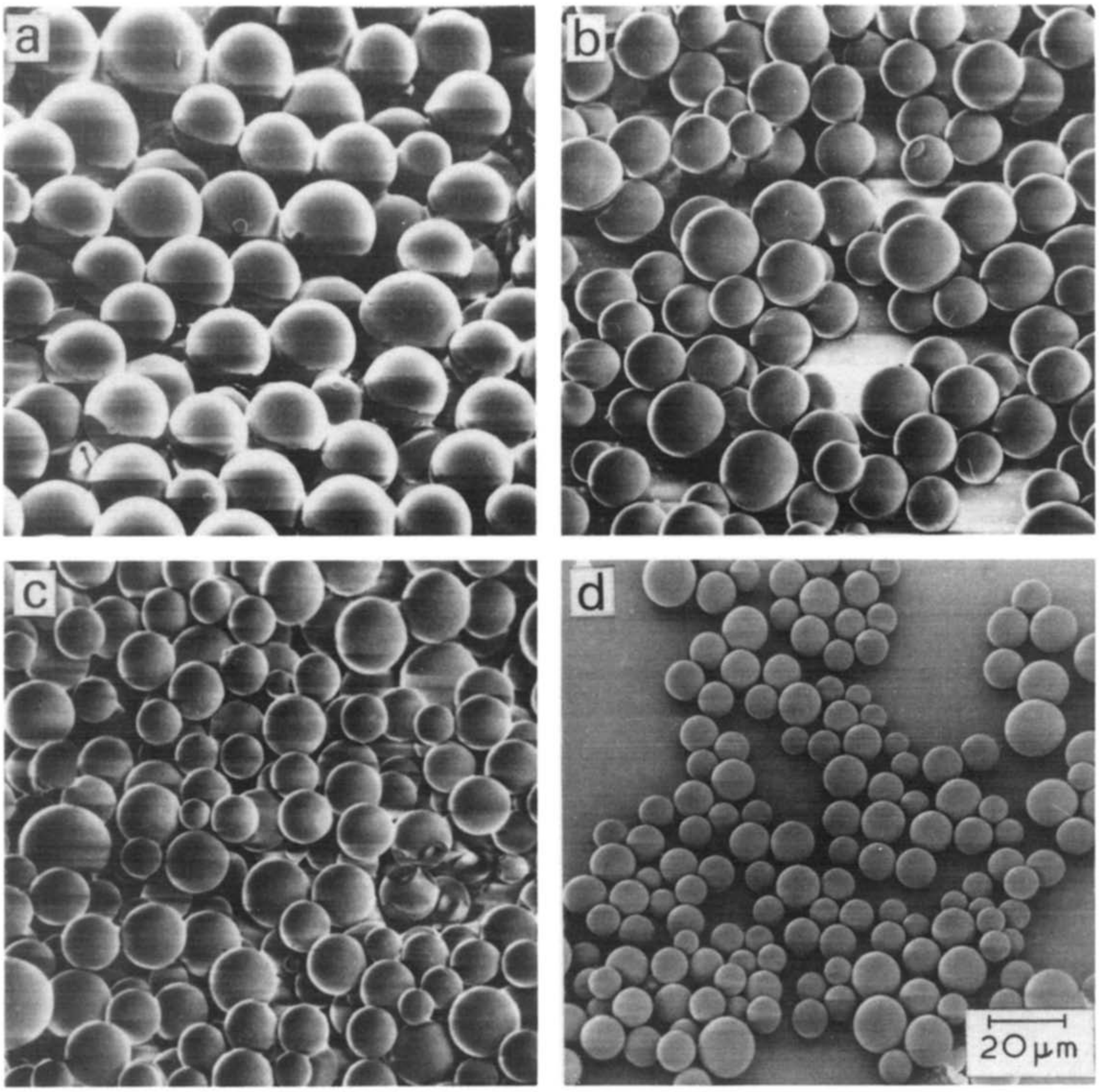

Figure 7 Scanning electron micrographs of gel particle fractions. (a) 24–28 μm; (b) 20–24 μm; (c) 16–20 μm; (d) 10–15 μm

Figure 6. It is clear from *Table 1* that the average diameter of the particles giving the results in *Figure 6* is close to the diameter of Spherisorb silica which consists of almost spherical particles having a narrow size distribution, see ref 14. Apart from increasing mobile phase dispersion, microparticulate packings with wide size distributions are undesirable experimentally. The presence of a substantial fraction of very small particles at the tail of a distribution produces a column of low permeability, necessitating high pressures in order to obtain reasonable eluent flow rates. Columns of Micrel (trade name of Polymer Laboratories Ltd) which is a microparticulate polystyrene gel have good permeability and performance[22].

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