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POROUS SILICA MICROSPHERES FOR HIGH-PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY

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SUMMARY

Rapid and precise high-performance gel permeation chromatography of polymers has been accomplished with columns of 6–9-μm trimethylsilyl-modified porous silica microspheres with narrow pore-size distributions over the range 60–3500 Å. A 60-cm, five-column combination of this pore-size range exhibited a plate count of $2.5 \cdot 10^4$ for a totally permeating solute (toluene) and 10^4 plates for a 10^5 molecular weight polystyrene. This column set has an overall fractionation range of $10^2 -> 7 \cdot 10^6$ for polystyrene, and its high resolution permits the determination of molecular weights in a 15-min analysis with an accuracy of about $\pm$ 2%, without peak dispersion corrections. The high resolving power of the microspheres, coupled with excellent mechanical strength and high purity, provides superior systems for rapidly characterizing both high-molecular-weight polymers and mixtures of small molecules by the size exclusion method.

INTRODUCTION

It has been known for some time that the efficiency of columns for high-performance liquid chromatography (HPLC) can be substantially improved by working with particles of <10 μm, providing a homogeneous packing structure is produced[1–7]. Very high column efficiency is a result of the rapid equilibrium (or rapid mass transfer) of solute molecules in a packed bed of such small particles, primarily because of the short diffusion distances involved. Studies have suggested that particles of *ca.* 7 μm provide a useful compromise between column efficiency and the practical aspects of HPLC[2–6].

Previous reports have defined the performance and general characteristics of <10-μm porous silica microspheres (PSM) primarily for liquid–solid and liquid–liquid chromatography[2,4,6]. These particles had surface areas of 350–50 m^2/g and pores of about 60–350 Å. More recently, packings of PSM particles with chemically bonded hydrocarbon phases have been described[8]. Actually, the PSM particles can be used for all four forms of HPLC (liquid partition, liquid–solid, ion-exchange, and size exclusion), since both the overall size and internal porosity of the particles can be selected and closely maintained. This paper describes the characteristics of deactivat-

ed, rigid, porous silica microspheres for the high-performance gel permeation chromatographic (HPGPC) analysis of organic macromolecules by size exclusion.

PSM particles are produced by the agglutination of collodial silica particles to form spherical microspheres of uniform diameter[9,10]. The particles are formed by reacting a mixture of urea and formaldehyde in the presence of a particular silica sol in an acidic aqueous medium. Formation of a liquid urea–formaldehyde polymer instigates the coacervation of the silica sol into microparticles. These particles filled with hardened organic polymer are then burned in air to form uniform-size, porous, pure silica microspheres. These particles consist of an array of uniform-size colloidal silica particles arranged in an interconnected three-dimensional lattice defining a system of internal pores having uniform and controlled dimensions. The size of these pores is determined by the size of the colloidal particles used to form the microspheres. The specific surface area of the PSM particles is essentially that of the silica sols from which they were formed, and the colloidal silica particles occupy no more than about 50% of the total volume of the microspheres, with the remaining volume occupied by the interconnected pores.

The use of *ca.* 6-μm particles with 350 Å pores for HPGPC was described briefly in the initial publication on the PSM particles[2]. This paper describes the characteristics of a variety of PSM particles in the 5–10-μm range, with individual preparations having narrow-distribution pore sizes ranging from 60 to 3500 Å and nitrogen surface areas of 330–8 m^2/g. These characteristics make the PSM particles particularly useful for characterizing macromolecules by HPGPC.

In modern gel permeation chromatography (GPC), the column efficiency parameter H, the height equivalent of a theoretical plate or HETP value, normally can be described as,

$$H = \frac{1}{(1/C_e\, d_p) + (1/C_m\, d_p^2\, u/D_m)} + \frac{C_{sm}\, d_p^2\, u}{D_m} \qquad (1)$$

The contribution for each of the terms in eqn. 1 to the plate height are functions of, (a) the particle diameter d_p of the column packing particles, (b) the mobile phase velocity u, and (c) the molecular diffusion coefficient D_m of the solute in the mobile phase. Coefficients C_e, C_m and C_{sm} are column constants.

From eqn. 1 we see that small H values, (which result in large column plate count N values and better separations) are favored for small column packing particles and for solute molecules having superior mobility. H is essentially dependent on the square of the particle diameter, but is a linear function of the reciprocal of the solute diffusion coefficient. Thus for macromolecules with small diffusion coefficients, plate heights will be larger and resolution will be poorer than for small molecules. On the other hand, plate heights for macromolecules will become increasingly smaller as the particle size is reduced, all other conditions equal. The effect of particle size is most important for very large molecules having very small diffusion coefficients, and use of columns with very small totally porous particles is particularly favored. Eqn. 1 indicates that a modest decrease in particle size, for instance, from 10 to 7 μm, results in a substantial decrease in plate height, in this case, by about a factor of two. The effect of particle size is even more important for very fast HPGPC separations at high mobile phase velocities.

Eqn. 1 suggests further improvement in column performance as particle size is decreased below 7 μm. However, there is currently a practical limitation in the size of particles in HPLC systems, since particles of less than about 5 μm are difficult to use in columns at maximum efficiency. Consequently, expected improvement in resolution often is not realized. In addition, column back-pressures increase substantially with decreased particle size (constant column length), which can be an experimental disadvantage. Lastly, it appears that <5-μm particles should be packed in very short columns (*i.e.*, $\leqslant 10$ cm) for good bed homogeneity. Unfortunately, short columns with small volumes are very susceptible to extracolumn band-broadening imposed by sample injection systems, column-connectors and detectors. This is of particular importance in HPGPC where peaks elute in very small volumes.

GPC traditionally has been carried out with columns of porous, cross-linked divinylbenzene–polystyrene gels with about 50-μm particles. More recently, columns of 10-μm gels have been made commercially available for GPC, with the predicted advantages in separation speed and resolution. While columns of these small, porous gels are satisfactory for some applications, they have serious disadvantages for many HPGPC uses because of mechanical limitations.

On the other hand, rigid particles have several significant experimental advantages over organic gels for HPGPC. They are relatively easily packed into homogeneous columns that are mechanically stable and not easily degraded with use. A much wider range of mobile phases may be used, resulting in greater versatility and increased convenience. The rigid packings rapidly equilibrate with new solvents, so that solvent changeover is fast and easy. Columns of rigid packings also are stable at higher temperatures with particular solvents required for characterizing certain macromolecules (*e.g.*, polyolefins) whereas, 10-μm particles of organic gels are used with great difficulty or may not be used at all with certain solvents under these conditions.

A potential disadvantage of the rigid packings is retention of solutes by adsorption. However, siliceous surfaces can be altered with certain organic functional groups to effectively eliminate this disadvantage for most applications.

MATERIALS AND METHODS

Apparatus

The HPLC apparatus and general technique used in this study have been described previously[2,4,6,8]. Samples were introduced into columns with a Model CV-6-UHPa-C-20 microsampling valve, (Valco, Houston, Texas, U.S.A.) with an external sample loop.

The column tubes used in this study were especially selected for highly polished inside walls. Experience has shown that the inside diameter of narrow-bore tubing should have a mirror finish, presumably because the walls influence the homogeneity of the packed bed produced by the high-pressure filtration column packing technique. Highly polished inside walls appear to have less of an effect in columns of greater than 0.4 cm I.D. containing less than 10-μm particles.

Most of the columns used in this study were made from precision-bore stainless-steel tubing manufactured by Superior Tubing (Norristown, Pa., U.S.A.). This tubing is described as Type-316L superpressure tubing with an inside mirror finish.

Mirror-finish inside walls for 0.635 cm O.D. × 0.46 cm I.D. columns were obtained with ordinary seamless stainless-steel tubing by polishing (Steel-Brite Polishing, Elizabeth, N.J., U.S.A.). Stainless-steel compression fittings (Swagelok; Crawford Fitting, Cleveland, Ohio, U.S.A.) were modified for minimum dead-volume and unobstructed flow-through patterns[4]. Column blanks were carefully cleaned before use[7].

Reagents and chromatographic particles

Chromatographic solvents were distilled-in-glass from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). PSM packings were prepared by the techniques previously described[9,10]. These particles were deactivated (silanized) by exhaustive reaction with chlorotrimethylsilane[8].

LiChrospher column packings were obtained from EM Labs. (Elmsford, N.Y., U.S.A.). To eliminate alkaline impurities, these materials were heated in concentrated nitric acid on a steam-bath for 2 h and washed to neutrality with distilled water. Large aggregates and "fines" which initially prevented the preparation of efficient columns were removed by sedimentation in 0.001 *M* ammonium hydroxide. The resulting material was again washed to neutrality with distilled water, filtered and dried at 150° for 2 h.

Polystyrene standards were from Pressure Chemical Co. (Pittsburgh, Pa., U.S.A.) and Duke Standards (Palo Alto, Calif., U.S.A.).

Column packing technique

Columns were prepared by the high-pressure filtration ("slurry") method, using the equipment and technique previously described[8]. Initially, columns were made with the balanced-density slurry packing technique[11]. Particles were suspended in mixtures of tetrabromoethane with various other solvents (*e.g.*, tetrahydrofuran, perclene) to obtain both a stabilized slurry and optimum particle dispersion. Tetrabromoethane was purified by passage through a 20 cm × 3.2 cm I.D. column of <20-mesh activated Davidson Type 62 silica gel (W. R. Grace, Baltimore, Md., U.S.A.) and stored overnight with a few drops of mercury.

Later in this study columns were prepared with methanol–chloroform (1:1) as the suspending liquid for the slurry packing technique. This is generally the preferred approach with PSM particles for HPGPC since it is more convenient, produces equivalent or better results compared with the balanced-density method, and is less hazardous than using toxic systems containing tetrabromoethane. Slurry-packed columns made by this technique which have been consolidated at high pressures[8], are very stable and show no evidence of settling after months of use with a variety of mobile phases at elevated temperatures and pressures.

Data handling and calculations

Data on column performance were obtained with a supplementary program on the DuPont Experimental Station PDP-10 real-time computer system[12,13]. Plate height and column plate count were derived from peak areas using the method suggested by James and Martin[14]. Mobile phase velocities were based on the elution of totally excluded polymers.

PARTICLE CHARACTERISTICS

The properties of some of the PSM particles used in this study are summarized in Table I. The PSM numbers given in Table I and throughout this paper refer to the average size of the silica sol used to prepare the specific particles. The notation "S" after the PSM number indicates particles exhaustively silanized with chlorotrimethylsilane. Particles with pore sizes intermediate to those in Table I also have been prepared but were not fully characterized by chromatography.

Particle size and particle size range

The particle size and the particle size range of PSM particles are controlled in the synthesizing reaction and subsequent sizing is not required. At the 7-μm particle size level it is usually possible to control the average size to better than about $\pm$ 1.5 μm, independent of porosity. For particular applications both larger and smaller particles have been prepared by varying appropriate reaction conditions.

Particle size distribution of each porosity of the porous silica microspheres is generally less than $\pm$ 20%, relative (1 σ), which is important for making reproducible, efficient columns. Because of the small size and narrow particle size distribution, fractionation of PSM by sedimentation during the high-pressure slurry packing procedure usually is not a problem, and the procedure may be performed satisfactorily without using a balanced-density slurry. The high efficiency of PSM columns is a result of the small particle size. The excellent reproducibility with which columns may be formed and the relatively high column permeability is a function of the spherical PSM particle shape and narrow size distribution.

Pore diameter

The PSM particles described in Table I have pore sizes ranging from 50 to 3500 Å, which is the range of general interest for size exclusion chromatography. The average pore size of the PSM particles is determined by selecting the colloidal silica particles used in their preparation —the larger the sol particles, the larger the pores. Since the agglutination of sol particles approximate a random close-packed structure of about 50% porosity, one can predict that the average diameter of the pores in the PSM particles should be roughly one-half the diameter of the sol particles making up the structure. As seen in Fig. 1 this relationship is verified by actual experimental values, except for very small sol particles. With very small silica sols the particles become strongly linked together in a more open network and the pore diameter is usually closer to the sol particle diameter. Thus, PSM particles formed from 50-Å sol have pores that are about the same diameter. PSM-4000 was not included in this plot since its preparation was carried out by a slightly modified process, resulting in some perturbation of the expected random close-packed structure.

Fig. 2 shows stero-scanning electron micrographs of some wide-pore PSM particles. Particles with smaller pores show increasing surface smoothness[4].

The distribution of pore sizes in the PSM particles is very narrow, approaching the limit predicted by theory for maximum resolution by size exclusion chromatography[15]. This narrow distribution is illustrated by the mercury intrusion plots in Fig. 3. PSM-800 has internal pores in 250-340 Å range with an average of about 300 Å. To insure a narrow distribution of pore sizes, a two-fold maximum size range

TABLE 1

CHARACTERISTICS OF POROUS SILICA MICROSPHERES

PSM design.	*Size** *(μm)*	*Ave. pore size (Å)*		*Nitrogen surface area (m^2/g)*		*Internal porosity (ml/g)*		*Porosity, volume percent*		*Linear fractionation range** ($\bar{M}_w$)*
		B.E.T.	*Hg*	*B.E.T.*	*Flow method*	*B.E.T.*	*Hg*	*B.E.T.*	*Hg*	
50	5.9 ± 1.3	60	60	326	271	0.658	0.389	59.1	47.2	$<10^2$–10^4
300	8.9 ± 2.2	237	125	87	84	0.514	0.384	53.1	45.8	$5\cdot10^3$–$4\cdot10^4$
500	7.7 ± 1.9	290	250	52	58	0.353	0.359	43.8	44.1	10^4–10^5
600	7.1 ± 1.7	330	195	45	52	0.296	0.325	—	41.7	10^4–$7\cdot10^4$
800	6.0 ± 1.5	—	300	34	32	—	0.426	—	48.3	$6\cdot10^3$–$2\cdot10^5$
1500	8.9 ± 1.2	—	750	—	20	—	0.459	—	50.2	$4\cdot10^4$–$2\cdot10^5$
4000	*ca.* 6***	—	3500	—	8.7	—	0.512	—	53.0	$7\cdot10^4$–$>7\cdot10^6$

* Quantimet analysis, $\pm1\sigma$, distribution weighted by volume.

** Polystyrene standards in tetrahydrofuran.

*** Semiquantitative optical microscopy.

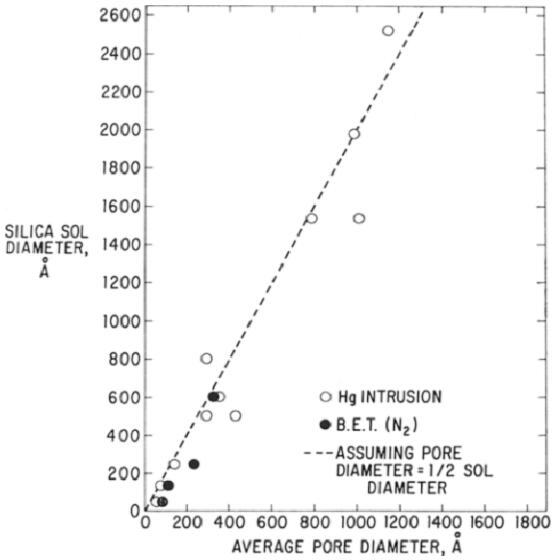


Fig. 1. Effect of silica sol diameter on PSM pore size.

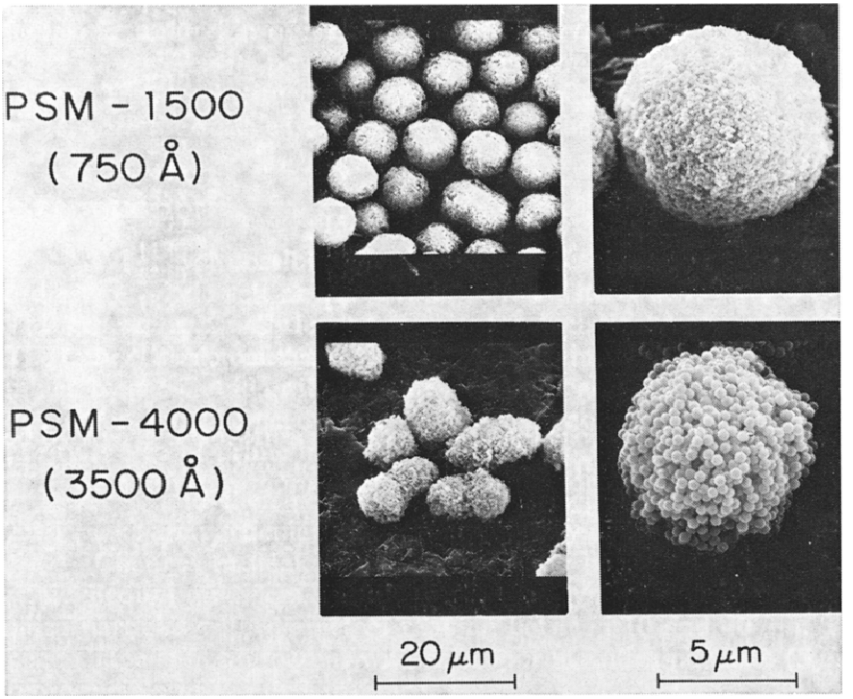


Fig. 2. Scanning electron micrographs of wide-pore porous silica microspheres.

was sought for the silica sol used to prepare the PSM particles. However, theory[15] and practice suggest that a four-fold range in sol size probably can be tolerated without radically affecting GPC resolution.

The mercury porsimetry measurement in Fig. 3 also provides insight into the mechanical strength of the PSM pore structure. PSM-800 particles show a significant break in the decreasing pressure or pressure relaxation plot at about 900 Å after a pressurization by mercury at 60,000 p.s.i. This effect suggests that the basic integrity of the PSM-800 internal pore structure remains after pressurization. The excellent mechanical strength of the PSM particles is presumably due to the rigid, random close-packing of the silica microparticles in the PSM structure. Particle stability is a desirable characteristic for the preparation of high-efficiency columns by the high-pressure slurry packing technique.

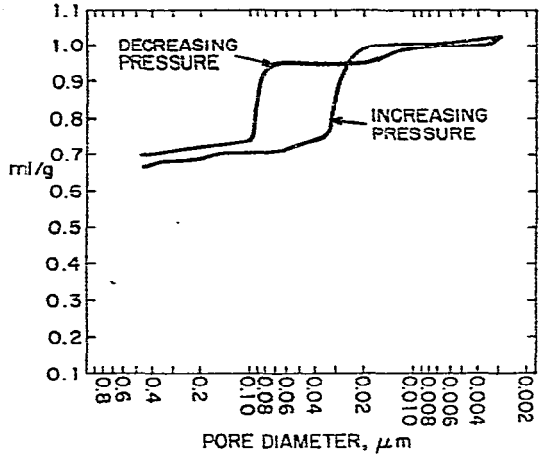


Fig. 3. Characterization of PSM-800 by mercury intrusion.

The average pore size indicated for the PSM particles in Table I is somewhat larger for nitrogen adsorption measurements (B.E.T.) than by mercury intrusion. This is typical of PSM particles and may be a characteristic of pore shape. One can speculate that the smaller values shown by mercury intrusion reflect the pressure needed to force mercury into pore segments whose dimensions are smaller than the average pore diameter. On the other hand, B.E.T. measurements are based on the total volume of nitrogen filling the pores, and may relate to the average diameter of the entire pore. The data suggest that the pores in the PSM particles may have narrow segments about one-half the average diameter of the bulk pore.

Pore volume and porosity

For maximum resolution in GPC a large pore volume is desired for the chromatographic particles[16]. However, it is proposed that the more pertinent parameter for use in size exclusion is not the specific pore volume, but the *volume porosity* or the volume of pores *per volume* of packing in a particular chromatographic column. The percent volume porosity V_v (ml/ml) can be calculated by,

$$V_v = 100 - [100/2.2\, V_p + 1] \qquad (2)$$

where V_p is the specific pore volume, ml/g. The data in Table I for the various PSM preparations confirm that the particles are composed of a random close-pack struc-

ture of approximately 50% pore volume. The effect of differences in specific pore volumes on GPC resolution is not as large as might first be imagined. For instance, compared with a column of particles with a specific porosity of 0.5 ml/g, an equivalent column of particles with a specific porosity of 1.0 ml/g shows only a 30% greater column fractionation volume (total permeation volume minus total exclusion volume). Of course, this is the result of the 0.5 ml/g material having a higher density —a greater weight is packed in the same column volume.

Some commercially available porous silica packings have a larger porosity than the PSM particles. On the other hand, with these commercially available silicas, porosity often decreases with increasing pore size. As shown in Table I, PSM particles have an essentially constant porosity throughout all pore sizes and particle sizes prepared.

PSM particles generally show the very narrow pore size distribution of about 0.2 (2 σ) in a conventional log-normal plot used in mercury porsimetry. Stated differently, the ratio of the pore sizes at the half-height of the pore-size distribution is *ca.* 1.6 (2 σ). This very narrow distribution results in calibration curves very close to that predicted for a single pore size. Column resolution in GPC is not substantially improved by using chromatographic packings with a narrower pore size distribution than the PSM particles because of the theoretical limits of the slope of the GPC calibration curve for a particular pore size[15]. Analysis suggests that a pore size distribution of about twice that of the PSM particles may be acceptable, without significantly reducing column resolution as result of increasing the slope of the GPC calibration curve[15].

Molecular weight calibration

Molecular weight calibration plots for columns of six different PSM preparations spanning the entire pore size range are shown in Fig. 4. The log of the molecular weight is plotted against the distribution coefficient, K, which is defined as,

$$K = (V_r - V_0)/(V_t - V_0) \tag{3}$$

where V_r is the retention time of the solute, V_0 is the retention volume of a totally excluded species, and V_t is the retention volume of a totally permeating species such as toluene. The data in Fig. 4 show the expected log dependence in the molecular weight of polystyrene standards as a function of K. All of the plots display linear portions, essentially parallel for all the pore size columns, shifting upwards along the log molecular weight axis in accordance with pore size increase. From the data in this figure, one can calculate the D_2 values (a function of the slope of the linear portion of the molecular-weight calibration curve) for each column using the equation,

$$D_2 = [\ln (M_2/M_1)/(K_1 - K_2)]\ [1/(V_t - V_0)] \tag{4}$$

where M_1 is the higher molecular-weight and M_2 is the lower molecular-weight species.

The columns characterized in Fig. 4 permit the analysis of solutes throughout a molecular weight range which exceeds that of many commercially available packings for GPC. For instance, column 1 (PSM-50; 60 Å) will fractionate solutes with a

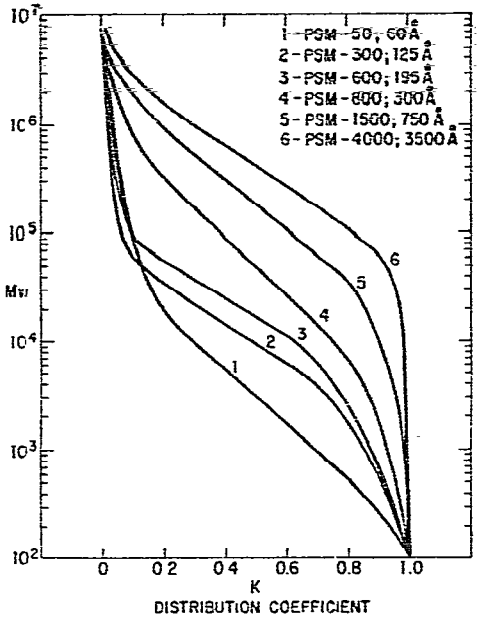


Fig. 4. Molecular weight calibration plots for PSM columns. Polystyrene standards; mobile phase, tetrahydrofuran, 22°; flow-rate, 2.5 ml/min; pressure, 925 p.s.i.; UV detector; columns listed in Table III; sample, 25 μl.

molecular weight of <100. At the other extreme, polymers of $>7 \cdot 10^6$ molecular weight are not yet totally excluded from column 6 (PSM-4000; 3500 Å).

Silanization of both narrow-pore and wide-pore PSM particles apparently causes no significant change in the molecular weight calibration plots for untreated particles, as indicated in Fig. 5 for polystyrene. This effect has been noted for columns of PSM particles throughout the entire pore size range.

Column efficiency

Since resolution in GPC is especially dependent on columns with large plate count, use of particles of less than 10 μm is particularly effective. Fortunately, as shown in later sections of this paper, even short lengths of *ca.* 7-μm particles produce sufficient plate count so that excellent resolution is obtained in polymer systems, resulting in small molecular-weight errors due to column dispersion effects[15].

Effect of mobile phase velocity

Columns of the small PSM particles (*ca.* 6 μm) show a very small increase in plate height with increasing mobile phase velocity, as illustrated in Fig. 6. Contrary to data from other porous silica particles for size exclusion[16,17], no differences in plate height were found for columns of untreated or silanized PSM particles. Also, no differences in plate heights were found for 10- and 15-cm columns of *ca.* 6-μm PSM-800 particles, confirming the homogeneity of the column beds for these different lengths with the column packing technique used. Both the untreated and silanized columns

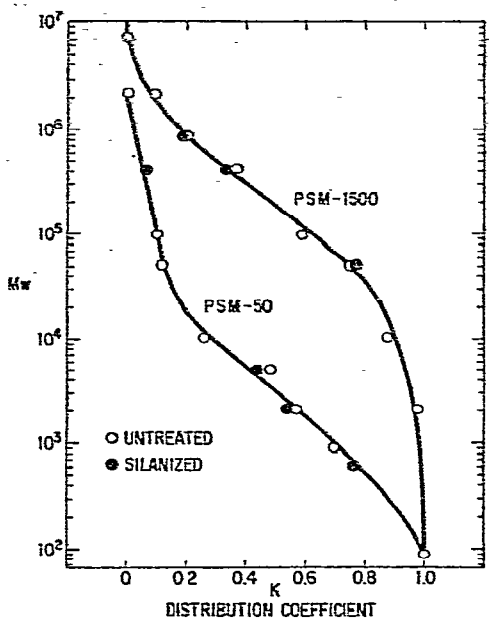


Fig. 5. Effect of particle silanization on molecular weight calibration curves. Conditions as for Fig. 4.

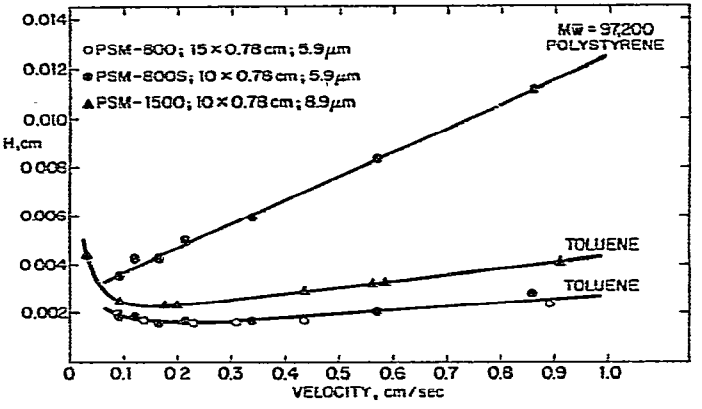


Fig. 6. Effect of velocity on plate height H for untreated and silanized PSM columns. Mobile phase, tetrahydrofuran, 22°, UV detector; sample, 25 μl.

show a reduced plate height, h, minimum of 2.5 particle diameters for the totally permeating toluene (equivalent to capacity ratio, $k' = 0$ in all methods except size exclusion). Similar results were obtained on columns of *ca.* 9-μm PSM-1500 particles.

Peak variance

Column efficiency also can be measured as a function of the peak standard deviation, σ, or as the variance of the peak, σ^2, calculated by,

$$\sigma^2 = t_R^2/N \tag{5}$$

where t_R is the retention time of the solute and N is the column plate count. Actually, in GPC the total observed peak variance is related by the expression

$$\sigma_t^2 = \sigma_c^2 + \sigma_d^2 \tag{6}$$

where σ_t^2 is the observed variance of the peak, σ_c^2 is the variance contribution due to normal peak broadening within the column, and σ_d^2 is the contribution of the peak variance caused by the molecular size fractionation of the polymer standard.

The relationship of the peak standard deviation σ_t as a function of polymer molecular weight for a series of high-performance PSM columns is shown in Fig. 7. If in the relationship shown in eqn. 6 the total observed peak variance σ_t^2 is dominated by the σ_c^2 column dispersion term, then one would expect a linear increase in the standard deviation of the peak σ_t with increasing molecular weight owing to the poorer mass transfer characteristics. (In eqn. 1, H increases essentially linearly with increasing molecular weight and decreasing diffusion coefficients.) This essentially linear (log) molecular weight *versus* σ_t relationship is observed for the PSM-800S column in Fig. 7. However, there is a significant divergence of some data from this relationship for the remainder of the columns. Columns of particles with small pores (*i.e.*, PSM-50S and PSM-300S) show very large σ_t values for lower-molecular-weight polymers. On the other hand, a generally linear relationship is obtained for toluene and the higher-molecular-weight solutes—it appears that the σ_d^2 values due to fractionation now are dominant contributors to the total peak variance σ_t^2, that is, the PSM-50S and PSM-300S columns partially fractionate the lower-molecular-weight, "monodispersed" polystyrene standards.

PSM-1500 and PSM-4000 particles with large pores show a similar effect with

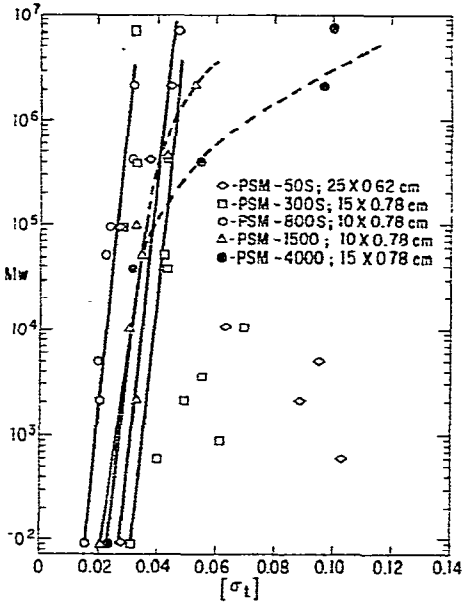


Fig. 7. Effect of molecular weight on peak σ_t for individual PSM columns. Conditions as in Fig. 4.

high-molecular-weight polymers for which these PSM particles show maximum resolution. For these large-pore particles a linear relationship between log molecular weight and σ_t is found with only lower-molecular-weight polymers, where the molecular weight fractionation term is small.

The contribution of the molecular-weight fractionation term is most observed with high-efficiency GPC columns with very small particles. Peak variance of conventional GPC columns of larger particles is largely determined by the column dispersion term, which is normally much greater than the molecular-weight fractionation term because of relatively low column resolution.

Effect of column internal diameter

A study of the effect of PSM column internal diameter was undertaken to determine the effect of this parameter in practical HPGPC. A series of 10-cm PSM-800 columns was tested with internal diameters ranging from 0.32 to 0.85 cm. The internal diameter *versus* plate height plot in Fig. 8 shows a minimum for these columns. Highest plate count and best peak symmetry were obtained with the 0.78-cm I.D. column, suggesting that this internal diameter is optimum for 10-cm columns of *ca.* 6-μm PSM particles. The optimum diameter of HPLC columns appears to be a complex function involving particle size, column length and internal diameter, sample injection technique, and other effects.

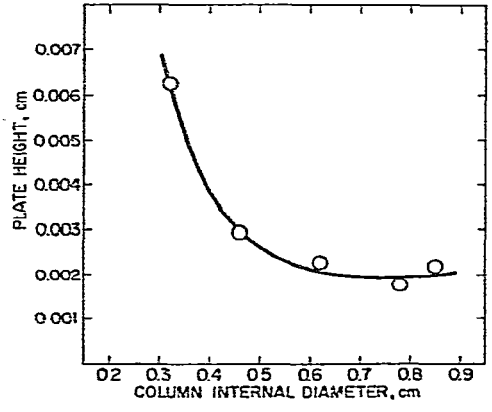


Fig. 8. Influence of column internal diameter on plate height. Columns, 10 cm, PSM-800, *ca.* 6 μm; sample, 6.7 μl, 1.0 mg/ml toluene; UV detector, 0.2 a.u.f.s.; mobile phase, tetrahydrofuran, 0.25 cm/sec.

COMPARISON WITH OTHER RIGID SMALL PARTICLES FOR HPGPC

During the course of this study, LiChrospher, another porous spherical particle of silica with various pore sizes, was made commercially available. To determine utility in HPGPC, these rigid packings also were studied. In Table II the relative usefulness of LiChrospher and PSM columns for characterizing polymers can be determined by comparing the packing resolution factor, R^*_{sp}, calculated by[15],

$$R^*_{sp} = \frac{0.576}{\sigma D_2 \sqrt{L}} \tag{7}$$

TABLE II

COMPARISON OF UNMODIFIED LICHROSPHER AND PSM PACKINGS FOR HPGPC

Packing	*Pore size (Å)*	*% Volume porosity*	*Particle diameter (μm)*	*Column dimensions (cm)*	*Velocity (cm/sec)*	*Pressure (p.s.i.)*	t_R *toluene*
LiChrospher 100	100*	72.5**	10 ± 2*	25 × 0.62	0.53	625	1.68
PSM-500	125	45.8	8.9 ± 2.2	25 × 0.62	0.53	700	1.37
LiChrospher 500	500*	63.7**	10 ± 2*	25 × 0.62	0.44	250	1.81
PSM-800	300	48.3	6.0 ± 1.5	25 × 0.62	0.49	1450	1.50
LiChrospher 1000*	1000*	63.7**	10 ± 2*	25 × 0.62	0.23	250	3.30
PSM-1500	750	50.2	8.9 ± 1.2	10 × 0.78	0.21	150	1.52
LiChrospher 4000	4000*	63.7**	10 ± 2*	25 × 0.62	0.20	400	2.79
PSM-4000	3800	53.0	6***	15 × 0.78	0.20	100	2.05

* Reported by manufacturer; PSM values from Table I.
** Calculated from eqn. 2 using specific pore volume given by manufacturer.
*** Semiquantitative optical microscopy.

where, σ, is the standard deviation of the test solute or polymer standard, D_2 is the slope of the linear portion of the log molecular weight *vs.* retention volume plot, and L is the length of column tested. These R^*_{sp} values are based on equal mobile phase velocities (equivalent analysis times) for a normalized (1 cm) column length[15]. For a valid comparison, particles with about the same average pore size were used. Columns of very small and large pore LiChrospher and counterpart PSM particles show essentially equal packing resolution factors for polymer standards. (R^*_{sp} values for the unretained solute toluene are less useful in defining the performance of packings.) However, in the mid-size pore range the PSM columns appear to exhibit higher resolution. Any resolution advantage of LiChrospher presumably is the result of greater selectivity (smaller D_2) because of a generally larger porosity, resulting in less-steep calibration plots. On the other hand, PSM columns show higher efficiency (smaller H and σ) because of smaller particles and a narrow particle size range, which provide superior packed beds of high specific permeability. The very narrow pore size distribution also contributes to the high resolution of the PSM packings. The effect of mass transfer on resolution due to differences in the pore structures for the two types of particles was not studied.

Fig. 9 illustrates the plate height advantage of the smaller PSM particles, particularly at the higher mobile phase velocities needed for very-high-speed HPGPC analyses.

Other results not shown in Table II have verified the utility of the R^*_{sp} factor for comparing packings using columns of different dimensions. For example, R^*_{sp} values for LiChrospher 1000 with a 50 × 0.48 cm column were 0.59 and 0.34 for toluene and 97,000 MW polystyrene, respectively, compared with the 0.61 and 0.41 values exhibited for the 25 × 0.62 cm column listed in Table II.

PSM particles are composed of very pure silica. The early lots of the commercial LiChrospher packings studied contained aggregates and alkaline impurities which required removal by extensive treatment. The effect of residual impurities on the analysis of certain polymers was not investigated.

Linear MW fractionation range	*σ (ml)*			D_2	*σD₂ (ml)*		R^*_{sp}	
	Toluene	*PS*			*Toluene*	*PS*	*Toluene*	*PS*
		MW	*σ*					
$3 \cdot 10^3$–$5 \cdot 10^4$	0.087	5000	0.229	1.28	0.111	0.293	1.05	0.39
$5 \cdot 10^3$–$4 \cdot 10^4$	0.067		0.147	2.17	0.145	0.320	0.80	0.36
$1.5 \cdot 10^4$–$1.5 \cdot 10^5$	0.107	51,000	0.275	1.32	0.141	0.363	1.01	0.32
$6 \cdot 10^3$–$2 \cdot 10^5$	0.054		0.118	2.25	0.122	0.226	0.95	0.43
$3 \cdot 10^4$–$2 \cdot 10^6$	0.096	97,000	0.142	1.98	0.190	0.281	0.61	0.41
$4 \cdot 10^4$–$2 \cdot 10^6$	0.030		0.068	4.56	0.140	0.308	1.30	0.60
10^5–>$7 \cdot 10^6$	0.092	390,000	0.144	3.84	0.353	0.553	0.33	0.21
$7 \cdot 10^5$–>$7 \cdot 10^6$	0.052		0.136	5.76	0.300	0.783	0.50	0.19

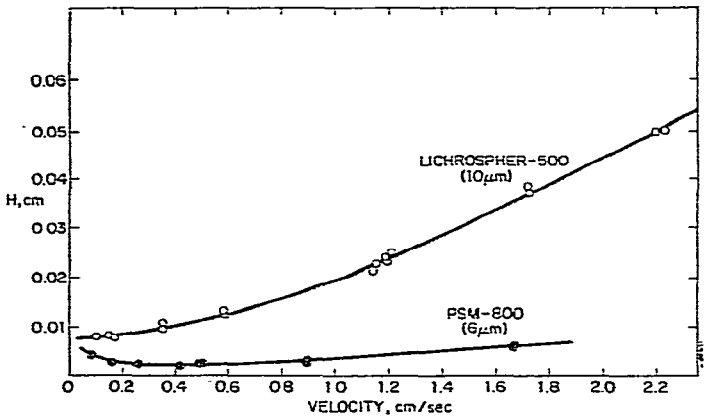


Fig. 9. Comparative plate height plots for LiChrospher and PSM columns. Columns, 25 × 0.62 cm; mobile phase, tetrahydrofuran; UV detector; sample, 25 μl, 5 mg/ml benzene in tetrahydrofuran.

PSM COLUMN SETS FOR HIGH-SPEED GPC

Table III summarizes the characteristics of individual working PSM columns in a full set prepared for HPGPC. The intent was to prepare each column with a plate count of about 4000, so that the set would have the total plate count required for accurate polymer molecular weight measurements[15]. All of the columns in this set contained silanized particles packed by the high-pressure filtration technique using dispersions in methanol–chloroform (1:1) pressurized at 5000 p.s.i. Packed bed consolidation techniques were utilized to insure mechanical stability of the structure[8]. The column packings were retained in the columns with 1.5-μm stainless-steel screens[4], and the columns were connected with 2-cm lengths of 0.05-cm I.D. capillary tubing with low dead-volume fittings.

TABLE III

SPECIFICATIONS ON INDIVIDUAL PSM COLUMNS FOR HPGPC

Flow-rate, 2.5 ml/min; particle sizes as in Table I.

PSM	*Column dimensions (cm)*	*Pressure drop (p.s.i.)*	*Velocity (cm/sec)**	*N toluene***	*H (cm)*	*h (H/dp) (at velocity shown)*
50-S	10 × 0.78	125	0.22	2972	0.00335	4.6
300-S	15 × 0.78	100	0.21	4000	0.00375	4.2
800-S	10 × 0.78	250	0.21	5535	0.00181	3.0
1500-S	10 × 0.78	210	0.25	3122	0.00320	3.4
4000-S	15 × 0.78	210	0.25	8145	0.00184	3.1

* Measured at total exclusion with 7,100,000 polystyrene (except not totally excluded in PSM-4000S).

** For total connected set, plate count = 23,900 (see text).

It was shown that plate count for the individual PSM columns listed in Table III are additive using the variance relationship from eqn. 5 in which,

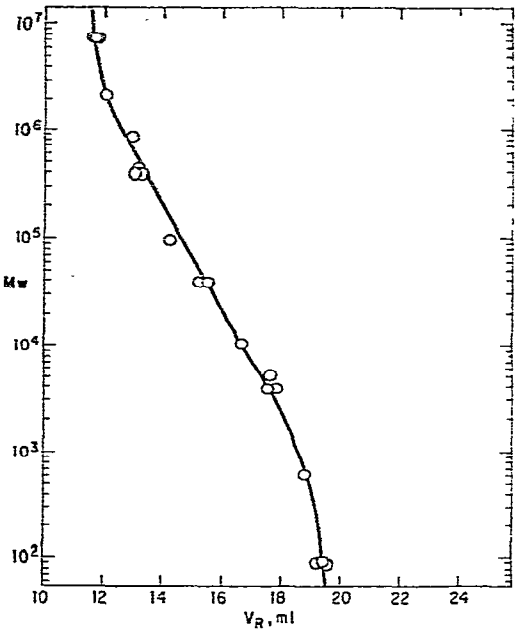
$$\sigma_t^2 = \sigma_1^2 + \sigma_2^2 + \cdots \sigma_n^2 \tag{8}$$

where σ_t^2 is the observed variance for the column set and σ_1^2, σ_2^2, and σ_n^2 are the individual variances for columns 1, 2 to n. (If the retention times, t_R, for the test solute are approximately equal for each column [$t_{R1} \approx t_{R2} \cdots \approx t_{Rn}$], then, $N_t = Y^2/\Sigma(1/N_i)$ where Y is the number of columns in series, N_t is the calculated total plate count, and N_i is the plate count for each individual column.) A total theoretical plate count for toluene of 21,500 was calculated for the column set using the relationship in eqn. 8, compared to 23,900 plates actually measured at a flow-rate of 2.5 ml/min. The slightly higher experimental plate count is probably due to reduced extra-column effects for the combined column.

A molecular-weight calibration plot for this five-column, 60-cm set of PSM columns is shown in Fig. 10. This set demonstrates a linear fractionation range for polystyrene standards of about 10^3–$2 \cdot 10^6$ MW, and a fractionation volume (total permeation minus total exclusion) of 7.9 ml. For a mixture of 97,200 and 10,300 MW monodispersed polystyrene standards, the specific resolution R_{sp} was 2.73 for this 60-cm set, compared with 1.14 for a 120-cm combination of μ-Styragel columns, both based on a 15-min analysis[15].

The very wide fractionation range of a set of PSM columns for polymers by HPGPC is predicted by the calibration plots in Fig. 10 and is further illustrated by the separation of polystyrene standards in Fig. 11. Note that the 7,100,000 MW polystyrene standard is not totally excluded. The high resolution of this PSM column set permits very accurate (*ca.* 2%) molecular-weight measurements in a 10–15-min polymer analysis without further correction for peak dispersion[15].

Fig. 12 shows the small plate heights exhibited by this PSM column set for various polystyrene standards, and particularly the relatively small increase in plate height with flow-rate (mobile phase velocity) increase. These data are indicative of the rapid solute equilibration associated with these very small silanized siliceous particles.



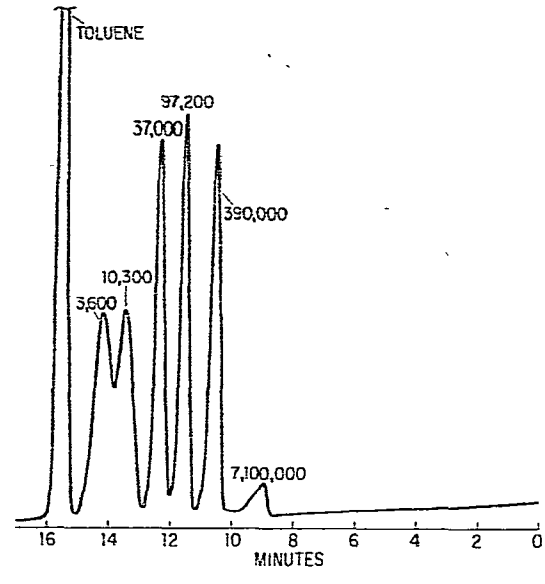
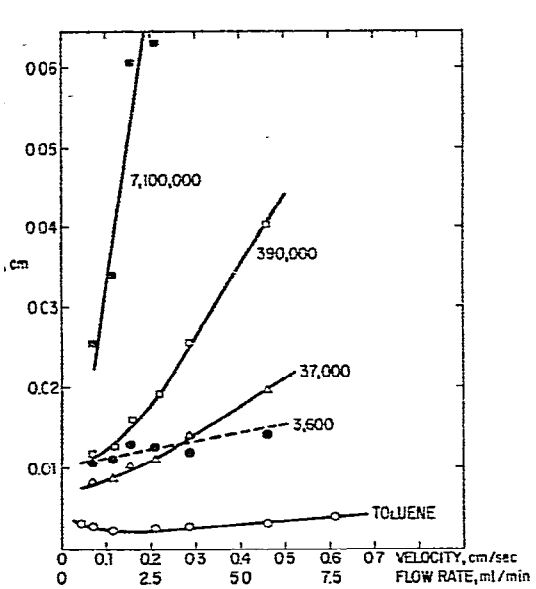


Fig. 10. Molecular weight calibration curve for full set of PSM-S columns. Columns: 10 cm PSM-50S, 15 cm PSM-300S, 10 cm PSM-800S, 10 cm PSM-1500S, 15 cm PSM-4000S (60 cm total, all columns, 0.78 cm I.D.); mobile phase, tetrahydrofuran, 22°; flow-rate, 2.5 ml/min; pressure, 925 p.s.i.; sample volume, 25 μl; polystyrene standards; UV detector at 254 nm.

Fig. 11. Separation of polystyrene standards. Conditions as in Fig. 10, except pressure, 425 p.s.i.; flow-rate, 1.25 ml/min; sample volume, 50 μl; UV detector, 0.1 a.u.f.s.

Mobile phase velocity can be substantially increased without significant sacrifice in resolving power for polymers because of the excellent mass transfer characteristics. Thus, columns of the PSM can be used for very fast, accurate polymer characterizations (*e.g.*, 90 sec[15]), such as might be required in a process analyzer monitoring a polymerization. Rapid analyses with a set of columns containing *ca.* 7-μm particles also can be carried out at relatively modest pressures (*e.g.*, <2000 p.s.i.) which are easily attained by modern pumping systems.

The shape of the plate height *versus* velocity plot for (totally permeating) toluene in Fig. 12 is as expected, with a plate height minimum at a velocity of approximately 0.15 cm/sec, representing about 25,000 theoretical plates for this system. (Tests with a 97,200 molecular weight polystyrene standard produced a plate count of 10,000 with this column set at a mobile phase velocity of 0.15 cm/sec.) The polymer standards show the anticipated decrease in plate height with decreasing velocity, but the less-steep slope of the MW = 390,000 and 37,000 polystyrene plots at the lower velocities was unexpected. One explanation is peak broadening from partial fractionation of these polymers by this high efficiency system. This trend is very apparent for the 3600 MW polystyrene (dotted line), which shows an anomalous, almost flat, plate height *versus* velocity plot, presumably because of significant molecular weight fractionation. Thus, because of partial fractionation of the polystyrene standards, measured plate height and σ values actually may be larger than true values. This



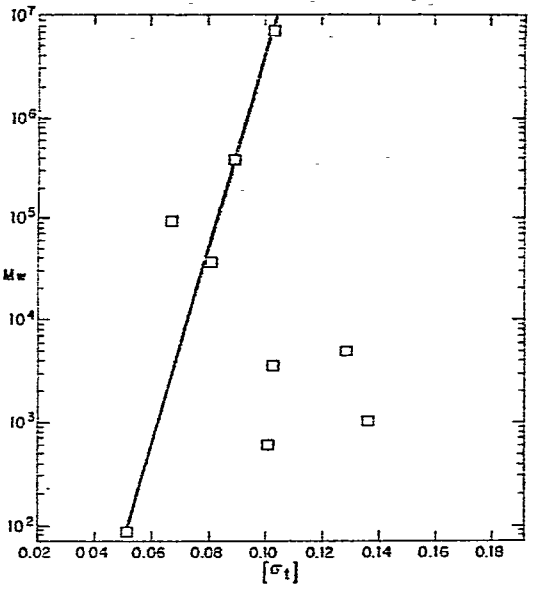


Fig. 12. Effect of mobile phase velocity on plate height for full set of PSM columns. Conditions same as for Fig. 10, except variable pressure and flow-rates.

Fig. 13. Effect of molecular weight on peak σ for full set of PSM columns. Conditions as in Fig. 10.

effect causes the predicted resolution of HPGPC columns to be less (*i.e.*, larger σD_2 values) than is actually the case.

Just as with individual PSM columns, the standard deviation of polymer peaks for the PSM column set is a function of molecular weight, as shown in Fig. 13. Much larger than predicted values were obtained for polymers of intermediate molecular weight because of molecular weight fractionation band-broadening by this high-efficiency system.

Columns of the modified PSM can be used with a variety of organic solvents (*e.g.*, methanol, acetone, dimethylsulfoxide) not possible with small particles of organic gels. Totally aqueous mobile phases should be avoided because of difficulty in wetting the silanized column packing. However, mixtures of water with the various miscible organic solvents can be employed if the particles are wetted. Aqueous mobile phases outside the pH 1–8.5 range should not be used because of possible degradation of the siliceous packing. In addition to these operational advantages, columns of PSM also have substantial advantage in resolution. A 60-cm set of PSM columns has demonstrated twice the resolution of a comparable 120-cm set of μ-Styragel columns, with accompanying advantage in molecular weight accuracy (*e.g.*, 2% *vs.* 13%[15]).

On determining the effect of mobile phase velocity on plate height, an anomoly was found with the 7,100,000 (7.1 MM) MW polystyrene. As shown in Table IV, the relative retention volume appears to change with increasing mobile phase velocity for the 7.1 MM MW polystyrene standard, but is not evident for lower-molecular-weight polymers. Collection of a 7.1 MM MW polystyrene peak chromatographed at *ca.* 0.5 cm/sec (5 ml/min) and re-injection into the column set at a mobile phase velocity of 0.1 cm/sec showed a lower molecular weight and broader-distribution

TABLE IV

EFFECT OF MOBILE PHASE VELOCITY ON RELATIVE RETENTION OF POLYSTYRENE STANDARDS

Flow-rate (ml/min)	*Approximate velocity (cm/sec)* *	V_R/V_R^0 *(PS/toluene)*			
		7,100,000	*390,000*	*37,000*	*3600*
0.50	0.05	0.567	0.680	0.796	0.910
0.76	0.07	0.569	0.678	0.794	0.910
1.28	0.11	0.580	0.679	0.795	0.909
1.78	0.15	0.592	0.681	0.796	0.909
2.50	0.21	0.609	0.683	0.796	0.910
3.39	0.29	**	0.684	0.799	0.909
5.26	0.46	**	0.680	0.793	0.912
7.27	0.61	**	0.684	0.794	0.917

* Based on 7.1 MM PS peak, not totally excluded.
** Not calculated; badly overlapping with 390,000 PS peak.

polymer. These data suggest that the 7.1 MM MW polystyrene is degraded at mobile phase velocities greater than about 0.1 cm/sec, probably as result of the mechanical shear forces being subjected on the large polymer chain under these conditions. A report that polystyrene of molecular weight above 10^7 shear degrades in a chromatographic system also has been given[18]. The changes in 7.1 MM MW polystyrene at higher mobile phase velocities also is in keeping with observations that this polymer is degraded by agitation of solutions in an ultrasonic bath.

These phenomena suggest that mobile phase velocities no greater than about 0.1 cm/sec should be used when characterizing very-high-molecular-weight polymers (*e.g.* MW $> 10^6$). Some polymers may be more subject to this effect than others.

Fig. 14 illustrates the use of a set of PSM columns for characterizing actual polymer samples in separate 12-min analyses of two cellulose samples as hemi-formal derivatives[19,20]. These chromatograms show that an experimental cellulose film lacks an additional high-molecular-weight component which is in a commercial cellulose film having different properties.

Retention of solutes beyond the total permeation volume is rarely exhibited by this deactivated silica set, suggesting that the coverage of the deactivating trimethyl-

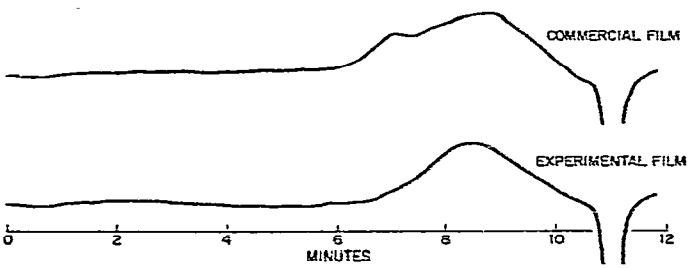


Fig. 14. High-speed GPC characterization of cellulose films. Columns, 10 × 0.78 cm of PSM-50S, PSM-800S, PSM-1500S and PSM-4000S (40 cm total); mobile phase, dimethylsulfoxide, 23°; pressure, 2000 p.s.i.; flow-rate, 1.0 ml/min; sample, 25 μl, 0.2% in dimethylsulfoxide–CH_2O; RI detector, 5 × 10^{-5} RI full scale.

silyl groups on the surface of the particles was adequate[8]. (The concentration of trimethylsilyl groups was 3.3 ± 0.4 μmole/m² for all PSM particles). Instances of retention beyond the total permeation volume probably can be attributed to a type of liquid-partition (or liquid–bonded-phase adsorption) retention caused by an inappropriate choice of mobile phase.

The small pore size and other desirable physical properties of PSM-50 (60-Å pores) makes this packing very useful for separating small molecules by size exclusion chromatography. Results on this application and in the use of PSM columns for separating water-soluble components by gel filtration chromatography will be described in future publications.

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