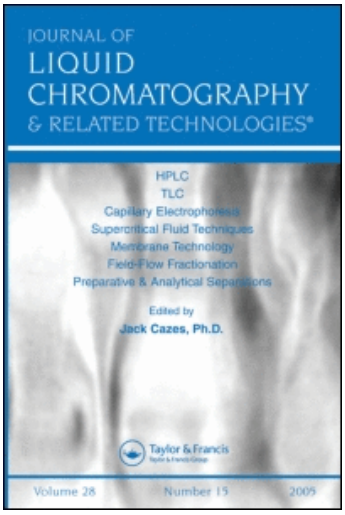

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REVERSED-PHASE CHROMATOGRAPHIC PROPERTIES OF MONODISPERSED MACROPOROUS PARTICLES OF POLY(STYRENE-DIVINYLBENZENE) PREPARED BY A MULTI-STEP SWELLING AND POLYMERIZATION METHOD

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ABSTRACT

Monodispersed macroporous poly(styrene-divinylbenzene) particles in a 5-6 μm size range were prepared by a multi-step swelling and polymerization method with toluene as the porogenic solvent, and their properties were examined. The particles were found to possess relatively broad pore size distributions. HPLC columns packed with the monodispersed particles show good efficiencies and low column pressure drops in a reversed-phase chromatographic mode.

INTRODUCTION

The growth of life science related separations has led to the development of various polymer-based packing materials in high performance liquid chromatography (HPLC) because silica-based packing materials are

less stable under the conditions required for the separations of biomolecules in some chromatographic modes. In most cases, polymer-based packing materials possess a much wider pH stability than silica-based packing materials which are generally best suited for applications between pH 2 and 8. Since their introduction in 1964 (1), the majority of polymer-based chromatographic packing materials have been based on styrene-divinylbenzene copolymers prepared by suspension polymerization (2,3). The polymerization technique is very important as it controls the ultimate properties of the final polymer. In suspension polymerization, monomers, a cross-linking reagent, and a polymerization initiator dissolved in a suitable organic diluent or porogen are stirred with water in the presence of a suspension stabilizer. The organic diluent is responsible for the formation of the porous structure and various kinds of pore size distributions can be obtained through changes in its nature. The polymerization proceeds in droplets of the organic phase under stirring. Unfortunately however, standard suspension polymerization technique results in particles having a broad particle size distribution that requires extensive size classification to meet the requirements of HPLC packing. Even after the tedious size classification process, the beads are not uniformly sized, and the presence of beads with different sizes degrades the column efficiency and leads to an increase in the column back pressure. Since the mechanical stability of the polymer-based packing materials is usually lower than that of silica-based packing materials, the high column back pressure leads to a short column lifetime, especially when used in a gradient mode with solvents that swell the beads since the column back-pressure changes as the composition of the mobile phase changes.

The resolution of a chromatographic column is greatly affected by factors such as its theoretical plate number, the distribution coefficient of the solutes, and the permeability of the packings. The solute permeability (4) is defined as the ratio of inner pore volume V_i to interstitial void volume V_0. Silica columns have permeability values ranging from 0.8 to 1.2. In order to increase the permeability of a column packing, it is possible to either decrease the void volume through enhancements in the packing process, or through the use of more porous, and consequently mechani-

cally less stable particles. Ideal packing requires that monodispersed particles be used; in this case, an idealized packing model of monodispersed spheres predicts that they will occupy at most 74.05 % of all available volume leaving 25.95 % as void volume V_0. However, this theoretical value can never be obtained in a real packed column. The best result described for a polymer packing was V_o = 34 % (5), in practice, most packings have at least 40% void volume.

A report by Ugelstad in 1979 described the preparation of uniform polymer particles using an activated multi-step swelling and polymerization method (6). However, the detailed preparation procedure has not been reported. This method has also been utilized for the preparation of the porous polymer particles that have been used recently for size exclusion chromatography (SEC) and ion exchange chromatography (IEC) (5,7).

We now report the detailed preparation and basic chromatographic properties of size monodispersed poly(styrene-divinylbenzene) particles in RPLC and SEC and compare their performance with that of macroporous polymer-based packing materials prepared by a more conventional suspension polymerization.

EXPERIMENTAL

Monodispersed poly(styrene-divinylbenzene) particles were prepared as follows; monodispersed polystyrene primary seed particles prepared according to a procedure described elsewhere (8) (7 ml, 7.2 weight % in water, particle size = 1.5 μm) were swollen with a solution of 0.3 g of benzoyl peroxide in 3.2 ml of dibutyl phthalate. In order to facilitate the swelling process consisting of transfer of solvent into the seed particles through the water phase, the solution of initiator in solvent was emulsified in 20 ml of water containing 0.15 g of sodium dodecyl sulfate (SDS) using a sonicator. The second swelling step was started after the total disappearance of the droplets from the previous emulsion. In the second swelling step, 16.5 ml of emulsified monomers (7 ml of styrene and 9.5ml of commercial divinylbenzene (55% DVB)), and 25 ml of tol-

uene (porogen) in 170 ml of water containing 3.4 g of poly(vinyl alcohol) (MW 85,000-146,000, 88 % hydrolyzed, Aldrich) were added to the dispersion resulting from the first swelling step. Polymerization was carried out in a 500 ml round-bottomed glass reactor (Buchi BEP 280) under continuous stirring (100 rpm) at 70 °C for 10 hours. After the polymerization was completed, the beads were transferred to a beaker containing 200 ml of methanol and decanted twice with methanol, then twice with THF. The yield of uniformly sized particles calculated with respect to the weight of added monomers was 95%.

For comparison purposes, similar porous styrene-divinylbenzene particles were prepared by a standard suspension polymerization technique. The organic phase consisting of 7 ml styrene, 9.5 ml divinylbenzene (55 % of DVB), 3.2 ml dibutyl phthalate and 0.16 g benzoyl peroxide was stirred in a 2 wt.% aqueous solution of the poly(vinyl alcohol) at 70 °C for 10 hours. The work up of the beads was done as described above.

Particle size distributions were measured with a sixteen-channel particle size analyzer, Coulter Counter Model TA II.

Chromatographic separations were carried out using a Nicolet LC9560 Ternary Gradient Liquid Chromatograph equipped with a Rheodyne 7125 valve loop injector (25 µm) and a column oven thermostated at 30°C. Peaks were monitored by an IBM 9563 Variable Wavelength UV Detector, at 254 nm for alkylbenzenes and polystyrenes and at 280 nm for proteins. The polymer particles were packed into a stainless steel column (4.6 mm ID x 150 mm) by the slurry method using constant flow mode with 80 % acetonitrile in water as the driving solvent. All solvents were of HPLC grade.

RESULTS AND DISCUSSION

Preparation of Porous Beads

The procedure we used in the multi-step swelling and polymerization method is a variation of that outlined by Ugelstad (6,7). It is easily adjusted for the preparation of monodispersed porous packings. Once suitable monodispersed seed particles are available (8) their size is readily

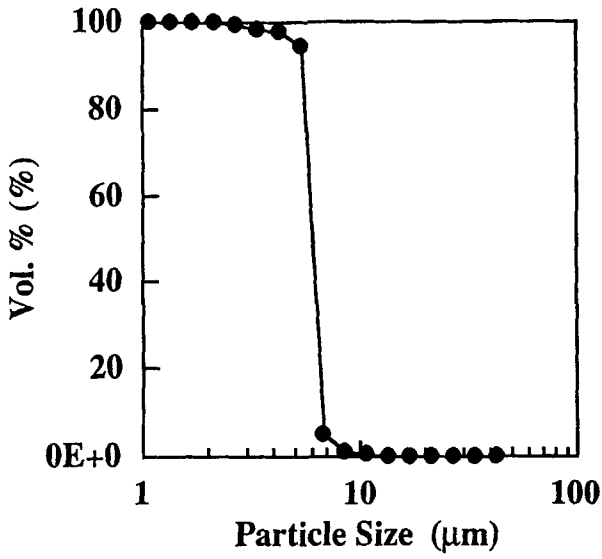

FIGURE 1. Particle size distribution of macroporous poly(styrene-divinylbenzene) particles prepared by the multi-step swelling and polymerization method.

modified for optimum applicability in HPLC. As the monodispersed seed particles are only able to undergo a few-fold increase in size in a single swelling step (8), their enlargement to the desired HPLC size has to be done in two steps. In the first step, a highly water-insoluble solvent such as dibutyl phthalate is utilized as an activating solvent which enables the further swelling step involving monomers and porogenic solvent. For example, the preparation described in the experimental section was developed to enlarge the 1.5 μm seed particles to a diameter of 6 μm. This represents a 64 fold increase in the volume of the seed particles.

Once the polymerization was completed, the mean size of the final particles was 5.6 μm as calculated from the particle size distribution curve shown in Figure 1. This size is slightly smaller than that of the swollen seeds (6 μm) due to the occurrence of shrinkage during polymerization. Examination of the beads by scanning electron microscopy confirms their size monodispersity.

Porosity and Pore Size Distribution

In this experiment the polystyrene of the seed particles and the activating solvent, dibutyl phthalate, only account for about 1 % and 7 %, respectively, of the total volume of the final swollen droplets. The volumes of porogen (toluene) and monomers used account for more than 55 % and 36 % of the total organic phase, respectively, which means that the porosity of the final particle obtained should be approximately 60 %. Two representative uniformly sized samples prepared by the two step swelling technique in this study had pore volume of 0.90 and 0.99 ml/g, which correlates well with the calculated porosity. Similar beads prepared by standard suspension polymerization had a pore volume of 0.86 ml/g. The presence of 1 % of polystyrene in the polymerization mixture in the last polymerization step constitutes the only difference between this multi-step swelling-and-polymerization method and the usual suspension polymerization method.

Size exclusion chromatography may be also used for the determination of the pore size distribution within porous beads (9). Figure 2 shows the calibration curves of beads prepared by the two-step swelling technique and by standard suspension polymerization. The absence of a sharp upper exclusion limit suggests a bimodal pore size distribution which is typical for polymeric stationary phases (10). The curves document the differences in pore size distribution for the two preparations. Typically, both types of beads have similar pore size distribution in the range of micropores and mesopores. The difference is observed in the range of macropores with sizes exceeding 50 nm, which are useful for the separation of polymers with molecular weight over 10^4. Therefore the beads prepared by the two-step swelling method provide better selectivity in the high-molecular weight region of the calibration curve. The volume of those macropores, amounting to approximately 10-15 % of total, was calculated from the calibration curve. As both, the uniformly sized and the standard suspension beads were prepared under almost identical conditions, the difference in the pore size distribution may be attributed to the presence of the polystyrene that constituted the original monodispersed seeds. The dissolved polymer of the seed particles acts as an additional

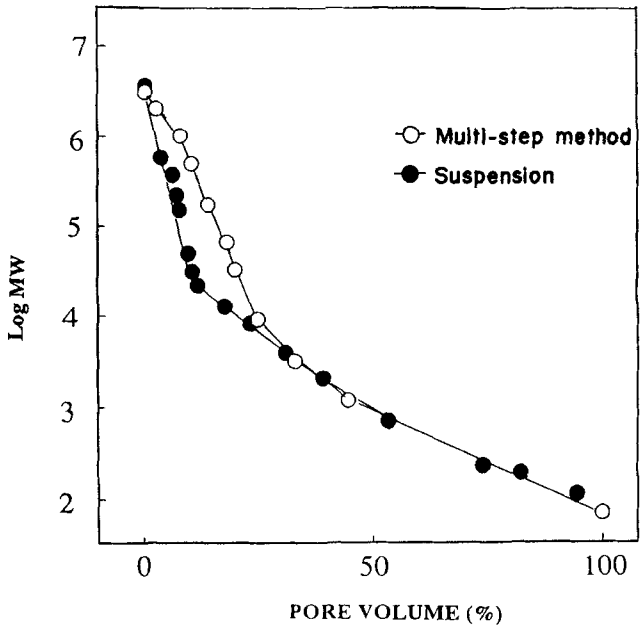

FIGURE 2. Calibration curves.
● Particles prepared by standard suspension polymerization method
○ Particles prepared by swelling method
Conditions: THF, 0.1 ml/min at 30°C; UV detector at 254nm.

porogen which causes broadening of the pore size distribution and shifts the maximum of the pore size distribution curve toward higher values (11).

Figure 3 shows the separation of polystyrene standards in THF using a column packed with monosized beads. Despite the short column length (150 mm) the separation in the low molecular weight region is quite acceptable. The resolution is somewhat poorer in the range of high molecular weights as the volume of the macropores is relatively small (10-15 %). The polystyrene standard with the highest molecular weight (2,100,000) apparently elutes at the total exclusion volume. The lack of separation ability in the range of medium molecular weights (34,500-68,000) is due to the bimodal pore structure of the beads (10).

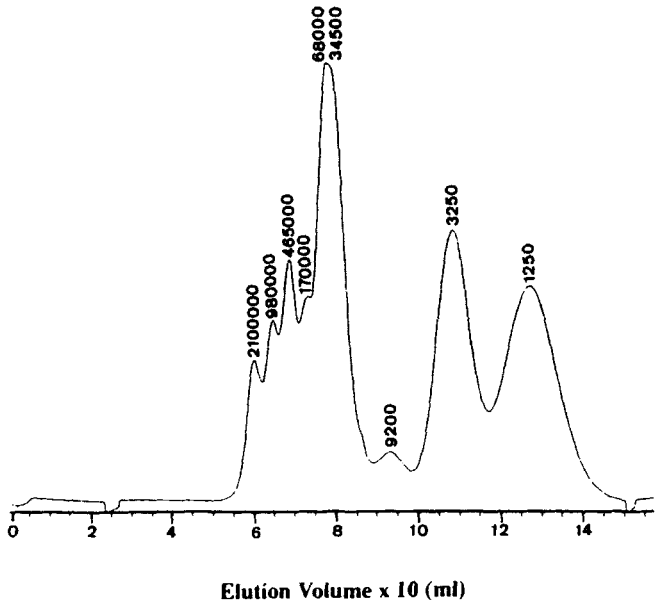

FIGURE 3. Separation of polystyrene molecular weight standards. Conditions: THF, 0.1 ml/min at 30°C; UV detector at 254nm.

Separation of Small Molecules in Reversed-phase Mode

Alkylbenzenes were separated to confirm the separation properties of the monodispersed beads in the reversed-phase mode of HPLC. Figure 4 shows a chromatogram of alkylbenzene separation obtained under isocratic elution with 80 % acetonitrile in water. The column efficiency calculated from the peak width amounts to well over 30,000 plates/m. It represents a reduced plate height approximately 5 times the bead diameter. This value is similar to that of beads PLRP-S 100 Å having a smaller size (12).

The pressure drop on the 150 mm column in the 80% aqueous acetonitrile solvent at 1 ml/min. was only 2 MPa, a value that is significantly lower than that observed for similarly sized particles prepared by suspension polymerization followed by size classification. For example, classified 2-7 μm beads have been found to give a pressure drop of 7.5

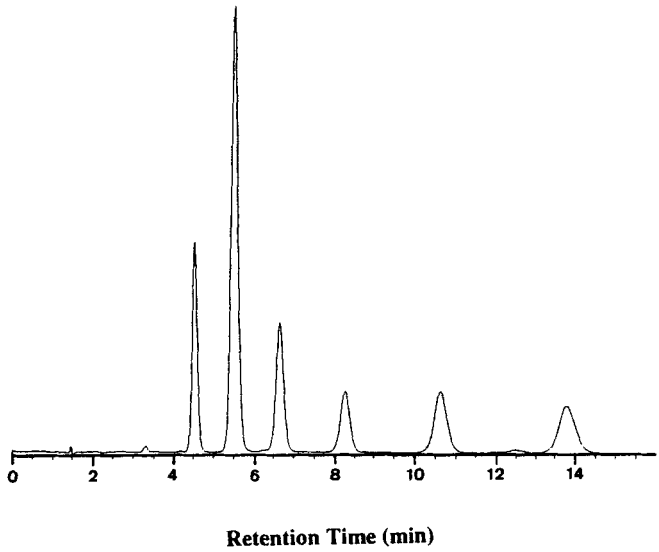

FIGURE 4. Separation of alkylbenzenes in reversed-phase mode. Conditions: H_2O-CH_3CN 4:1; 1 ml/min at 30°C; UV detector at 254nm. Samples: (in order of elution) benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene.

MPa at a flow rate of 0.8 ml/min (13). It is especially important to achieve a low pressure drop in the case of aqueous mobile phases, because this medium leads to pressure drops that are higher than those observed with the non-aqueous mobile phases utilized in a normal-phase mode. This is critical in the separation of biomolecules in an aqueous gradient mode and the change of pressure drop that results from changes in the composition of the mobile phase can be a very serious problem for mechanically less stable polymer-based packing materials. It is expected that initially high column pressure drops should lead to even larger changes in the pressure drop during a gradient cycle than low column pressure drops. When the column pressure drop is as low as is observed with our monodispersed particles, the use of smaller size particle can be contemplated in order to achieve higher column efficiencies. In addition, a higher flow rate can be applied to achieve a faster analysis.

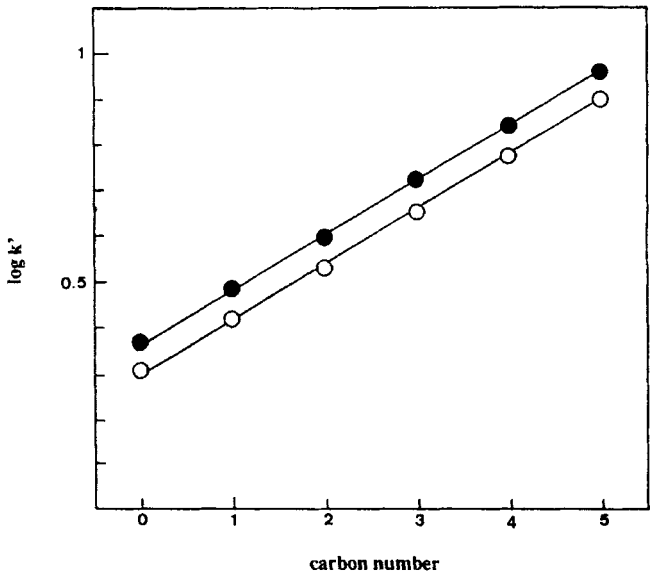

FIGURE 5. Log k' *vs.* carbon number of the substituent on benzene moiety in RP mode.
● Particles prepared by standard suspension polymerization method
○ Particles prepared by swelling method

A comparison of the chromatographic behavior of the monodispersed beads with size classified particles prepared by suspension polymerization reveals the better chromatographic characteristics of the monodispersed beads. For example, peak tailing, which is frequently observed (10, 14) in the separation of low molecular weight compounds using polymeric packings, is not a problem. As can be seen in Figure 4, the monodispersed beads show symmetrical peaks (As = 1.03) with no tailing, providing for a very high resolution. This is in sharp contrast to the behavior of several packing materials prepared by suspension polymerization (15,16).

Figure 5 shows the relationship between the number of alkyl C atoms in alkyl benzenes and log k' values to illustrate the retention performance of the particles in comparison to that of classified particles

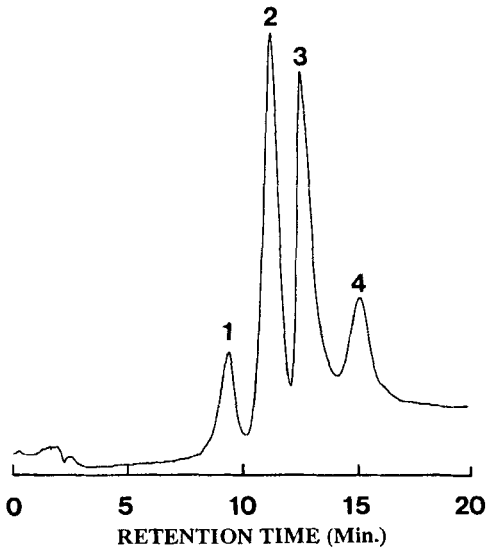

FIGURE 6. Separation of proteins. 1, Ribonuclease A; 2, Cytochrome C; 3, Lysozyme; 4, BSA; Conditions: Mobile phase A, 20% acetonitrile (0.1% trifluoroacetic acid); mobile phase B, 60% acetonitrile (0.1% trifluoroacetic acid; gradient from 100% A to 100% B in 20 min. 1 ml/min at 30°C; UV detector at 280nm.

prepared by suspension polymerization. Since in a reversed-phase mode, chromatography can be achieved mainly by a partition between mobile phase and stationary phase, the value of log k' for a homologous series of compounds is usually proportional to the number of C atoms in the alkyl chain. Therefore, as both lines shown in Figure 5 have almost the same slope, it may be assumed that both particles have similar hydrophobicities. The same selectivity value, $\alpha = 1.33$, for amylbenzene/butylbenzene obtained for both columns suggests that both particles have similar surface areas.

Separation of Proteins in Reversed-phase Mode

Wide pore packings have generally been utilized for the separation of proteins in a gradient elution mode. Figure 6 shows a separation of four

proteins, i.e. ribonuclease A (MW 13,683), cytochrome C (MW 12,300), lysozyme (MW 14307), and bovine serum albumin (MW 69,000), using a gradient of acetonitrile in water. The separation proceeds according to the hydrophobicity of the proteins and their size should not influence the separation. Unfortunately, even the monodispersed beads contain small pores too in which the protein diffusion is restricted and this causes some peak broadening.

CONCLUSION

The multi-step swelling and polymerization method can afford useful packing materials for HPLC with excellent size monodispersity and broad pore size distributions. These particles are efficient in reverse-phase mode and size exclusion mode as demonstrated in the separation of alkylbenzenes as well as synthetic organic polymers and proteins.

While this report concerned particles prepared under standard swelling and polymerization conditions, appropriate changes in the composition of the swelling systems can afford particles with a variety of different characteristics. We are currently developing new approaches that afford monodispersed particles with even larger pores, as well as particles with radial gradients of hydrophilicity for specialized separations.

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