

Monodisperse-Porous Poly(*Styrene-co-Divinylbenzene*) Beads Providing High Column Efficiency in Reversed Phase HPLC

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ABSTRACT: A multistage polymerization protocol, the so-called “modified seeded polymerization,” was developed for the production of monodisperse-porous poly(*styrene-co-divinylbenzene*) providing high column efficiency as a packing material in reversed phase high performance liquid chromatography (RPLC). In the first stage of the multistage production, uniform polystyrene seed particles, produced by dispersion polymerization, were swollen by an organic agent (i.e., the diluent) and then by a monomer mixture containing styrene and divinylbenzene. The final porous particles were obtained in the monodisperse form by the polymerization of monomer mixture in the seed particles. By the use of a small size seed latex with low molecular weight and by the selection of the appropriate diluent, relatively small monodisperse-porous particles with suitable pore structure could be achieved. In the reversed phase separa-

tion of alkylbenzenes, under isocratic conditions, theoretical plate numbers up to 40,000 plates/m were achieved by using 5.2 μm porous particles, obtained by a toluene-dibutyl phthalate mixture as the diluent. No significant decrease in the resolution power was observed by the fourfold increase in the mobile phase flow rate. The column efficiency and the resolution observed with 5.2 μm monodisperse-porous particles were significantly higher with respect to the currently available polymer based packing materials used in the reversed phase HPLC. © 2005 Wiley Periodicals, Inc. *J Appl Polym Sci* 95: 1430–1438, 2005

Key words: HPLC; reversed phase chromatography; chromatographic packing; divinylbenzene; porous particles; monodisperse particles; column efficiency

INTRODUCTION

Various methods have been proposed for the production of monodisperse porous polymer particles suitable as HPLC packing materials.^{1–22} The first method, the so-called “Activated Multi-Step Swelling and Polymerization,” was developed by Ugelstad et al. for the production of compact or porous-monodisperse particles in the range of 1–20 μm .^{1–5} Monodisperse-porous poly(*styrene-co-divinylbenzene*) particles 10 μm in size were also prepared by “Seeded Emulsion Polymerization.”^{6,7} “Staged Shape Template Polymerization” was another multistage protocol developed for the production of monodisperse poly(*glycidyl methacrylate-co-ethylene dimethacrylate*) particles.^{8,9} By starting from the polystyrene seed particles produced by dispersion polymerization, Ogino et al. established a single stage swelling and polymerization method for the production of poly(*styrene-co-divinylbenzene*) beads in the size range of 4.1–7.5 μm and

oligo(*ethylene glycol*) dimethacrylate beads in the range of 5.5–9.7 μm .^{10,11} These beads were used as packing material in Size Exclusion Chromatography (SEC) and exhibited good separation performance.^{10,11} Ogino’s approach was also modified for the preparation of monodisperse beads suitable for hydrophobic interaction chromatography (HIC) and ion-exchange chromatography (IEC).^{12,13}

In HPLC applications, the column efficiency increases with decreasing particle size of the packing material. Hence it is possible to perform chromatographic analysis by reducing the volume of the mobile phase in a shorter time. Hosoya et al. used a modified multistage polymerization method for the synthesis of poly(*2-ethylhexyl methacrylate-co-glycerol dimethacrylate*) beads with an average size of approximately 4 μm .¹⁴ The produced beads exhibited an excellent chromatographic performance in the separation of alkylbenzenes in semimicro HPLC and column efficiencies up to 13,000 plates were obtained in a column 15 \times 2.1 mm id.¹⁴ Recently, Zhang et al. developed a single stage dispersion polymerization process for the production of poly(*glycidyl methacrylate-co-divinylbenzene*) beads 3 μm in size.^{15,16} The quaternary am-

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TABLE I
The Properties of Polystyrene Seed Latices

Seed latex type	SL6	SL7
Polymerization yield (% w/w)	85	69
Average particle size (D_{nr} , μm)	2.1	4.4
Coefficient of variation (%)	1.6	2.0
Average molecular weight (M_w)	1.02×10^5	2.16×10^4

monium and sulfonic acid carrying forms of these beads were successfully used as packing material both in micro-HPLC and capillary electrochromatography (CEC).^{15,16}

We also proposed a multistage polymerization protocol, "modified seeded polymerization," for the synthesis of monodisperse particles. Poly(styrene) particles, obtained by dispersion polymerization with suitable molecular weight, were directly utilized as the seed latex in the production of porous particles.^{23,24} Hence, monodisperse particles with different chemical structures were obtained without applying any additional swelling stage for the synthesis of the polymeric part of the porogen. The method was also applied to the production of monodisperse porous particles carrying different functional groups like hydroxyl, carboxyl, epoxypropyl, and boronic acid.²⁵⁻²⁹ Recently, monodisperse porous poly(styrene-co-divinylbenzene) particles (7.5 μm in size), produced by "modified seeded polymerization," were successfully tried as packing material in HPLC.^{30,31}

In this study, we made some modifications to our procedure for reducing the size of porous particles. Hence, we aimed to obtain higher column efficiency in the chromatographic separations. To synthesize relatively small monodisperse particles, with suitable porosity properties, we started with a seed latex 2.1 μm in size and used a diluent mixture including both toluene and dibutyl phthalate in the multistage polymerization protocol. Then, monodisperse porous poly(styrene-co-divinyl benzene) particles 5.2 μm in diameter were synthesized. Until today, although polymer based monodisperse beads with relatively smaller size ($<5 \mu\text{m}$) have been synthesized with different methods, no attempt has been made to use these beads as packing in reversed phase HPLC

(RPLC).^{10,11,14-16,32} For this reason, monodisperse poly(styrene-co-divinylbenzene) beads, with relatively smaller size, were first tried as packing material in RPLC by this study. We wish to report the chromatographic performance of this material comparatively with the larger particles.

EXPERIMENTAL

Materials

Toluene and dibutyl phthalate (DBP) (both from Aldrich Chem. Co.) were the diluents used in the preparation of monodisperse-porous particles. Divinylbenzene (55% para and meta-divinylbenzene isomers, Aldrich Chem. Co.) was extracted with 5% (w/w) NaOH solution. Benzoyl peroxide (BPO, Aldrich Chem. Co.) was the oil-soluble initiator in the repolymerization of the monomer phase in the swollen seed particles. Sodium dodecyl sulfate (SDS, Sigma Chem. Co.) was the emulsifier in the preparation of the aqueous emulsion medium for the swelling of seed particles. Acetonitrile and tetrahydrofuran (THF)—both HPLC grade—were supplied from Aldrich Chem. Co. The suppliers and properties of materials used in the preparation of seed latices were given elsewhere.^{30,31}

Preparation of seed latices

Two types of seed particles were obtained by the dispersion polymerization of styrene.^{33,34} In the synthesis of seed latex 2.1 μm in size, styrene (10 mL), polyvinylpyrrolidone (PVP K-30, M_r : 40,000, 1.0 g), and azobisisobutyronitrile (AIBN, 0.10 g) were dissolved in ethanol (100 mL). The polymerization was conducted at 70°C, with a 120 cpm shaking rate for 24 h, in a sealed, cylindrical polymerization reactor (volume: 410 mL) placed in a shaking water-bath. For the synthesis of the seed latex 4.4 μm in size, styrene (20 mL), PVP K-30 (2.1 g), and AIBN (0.44 g) were dissolved in a solution comprised of ethanol (84 mL) and 2-methoxyethanol (36 mL). The polymerization was conducted under the same conditions described above.

TABLE II
The Production Conditions of Monodisperse-Porous Particles

Code	Seed latex size (μm)	Seed latex (g)	DBP (mL)	Toluene (mL)	S (mL)	DVB (mL)	BPO (g)	PVA (g)
SD1	2.1	0.26	2.4	—	0.6	2.0	0.12	0.35
SD2	2.1	0.26	—	2.4	0.6	2.0	0.12	0.35
SD3	2.1	0.26	1.2	1.2	0.6	2.0	0.12	0.35
SD4	2.1	0.16	1.2	1.2	0.6	2.0	0.12	0.35
SD5	4.4	0.55	2.0	—	0.6	2.0	0.12	0.35

TABLE III
The Average Size and Size Distribution Properties of Monodisperse-Porous Particles

Code	Seed latex size (μm)	Diluent	D_n (μm)	CV (%)
SD1	2.1	DBP	5.3	2.1
SD2	2.1	Toluene	5.0	3.0
SD3	2.1	Toluene/DBP	5.2	3.4
SD4	2.1	Toluene/DBP	6.1	2.4
SD5	4.4	DBP	7.8	2.5

Synthesis of monodisperse-porous particles

In the synthesis of relatively smaller porous-monodisperse particles, polystyrene (PS) seed latex 2.1 μm in size was used. Typically, the selected diluent (i.e., DBP, toluene, or DBP-toluene mixture, 2.4 mL) was emulsified in an aqueous medium (30 mL) including 0.25% (w/w) SDS. For this purpose, the mixture of diluent-SDS solution was sonicated for 30 min. The dispersion including PS seed particles (approximately 2 mL, solid content: 0.26 g) was added to the emulsion. The new dispersion was stirred magnetically (400 rpm) at room temperature for 24 h for the absorption of the diluent phase by the seed particles. In the following step, a monomer phase, comprised of styrene (0.6 mL), DVB (2.0 mL), and BPO (0.12 g), was emulsified in the aqueous medium (30 mL) including 0.25% (w/w) SDS by sonication for 5 min. Monomer emulsion was mixed with the emulsion containing diluent-swollen seed particles. The resulting emulsion was stirred at room temperature for 24 h at 400 rpm for the absorption of the monomer phase by the diluent-swollen seed particles. At the end of this period, an aqueous solution (3.5 mL) including 0.35 g PVA was added into the resulting dispersion. In the next stage, the emulsion was purged with nitrogen for 5 min. Repolymerization of the monomer phase in the swollen seed particles was carried out at 70°C and 120 cpm shaking rate for 24 h. Porous, monodisperse particles, approximately 5 μm in size, were obtained as the product.

Larger porous-monodisperse particles (8 μm) were also produced as a reference material by starting from a large seed latex (4.4 μm) and following a similar route. After repolymerization, the particles isolated by centrifugation were washed with ethanol several times by applying a centrifugation-decantation protocol, and finally extracted by THF according to the protocol described elsewhere.^{30,31} Scanning electron microscopy (JEOL, JEM 1200EX, Tokyo, Japan) was used for the determination of size properties (i.e., the average size, D_n , and the coefficient of variation, CV %) and the evaluation of surface morphology of the particles.^{30,31}

Chromatographic study

The particles were slurry packed into 150 mm \times 4.6 mm I.D. stainless steel HPLC columns according to the protocol given elsewhere.^{30,31} Chromatography was carried out using a Shimadzu gradient liquid chromatograph (LC-10 ADVP) equipped with a SPD-10 AVVP UV detector. Size-Exclusion Chromatography (SEC) was performed in THF with the flow rate of 0.75 mL/min at room temperature. The chromatograms were obtained by an SPD-10 AVVP UV detector at 254 nm. The distribution coefficient, K_o , was calculated based on eq. (1), where V_a is the retention volume of the selected alkylbenzene, and V_{toluene} and $V_{5,000,000}$ are the retention volumes of toluene and the PS standard with an average MW of 5,000,000, respectively.

$$K_o = (V_a - V_{5,000,000}) / (V_{\text{toluene}} - V_{5,000,000}) \quad (1)$$

The reversed phase separation of the alkylbenzene mixture (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene) was investigated under isocratic conditions by using an acetonitrile-water mixture as the mobile-phase. The liquid chromatograms were recorded by a UV-detector operated at 254 nm at different flow rates changed between 0.5–2.0 mL/min. The resolution of successive peaks and the theoretical plate number were calculated based on the usual expressions given in our previous publications.^{30,31}

The reduced plate height (h_r) of each column is calculated based on the following equation, where N_t (plates/column) is the theoretical plate number, L (m) is the column length, and d_p (m) is the particle diameter.

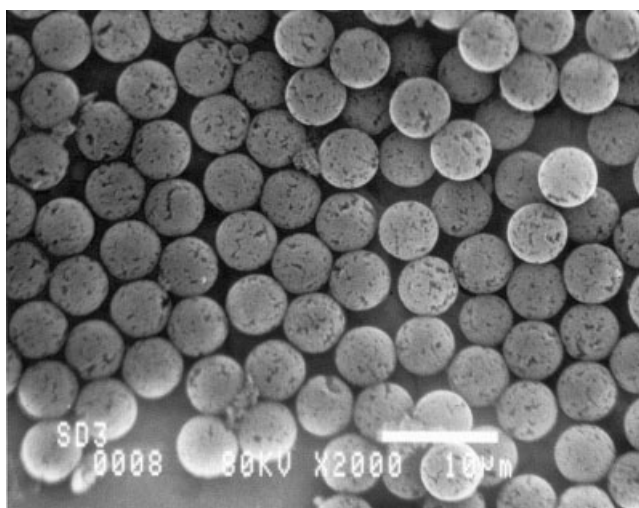


Figure 1 A representative SEM photograph showing the size and size distribution of porous particles encoded as SD3. (Magnification: 2000 \times .)

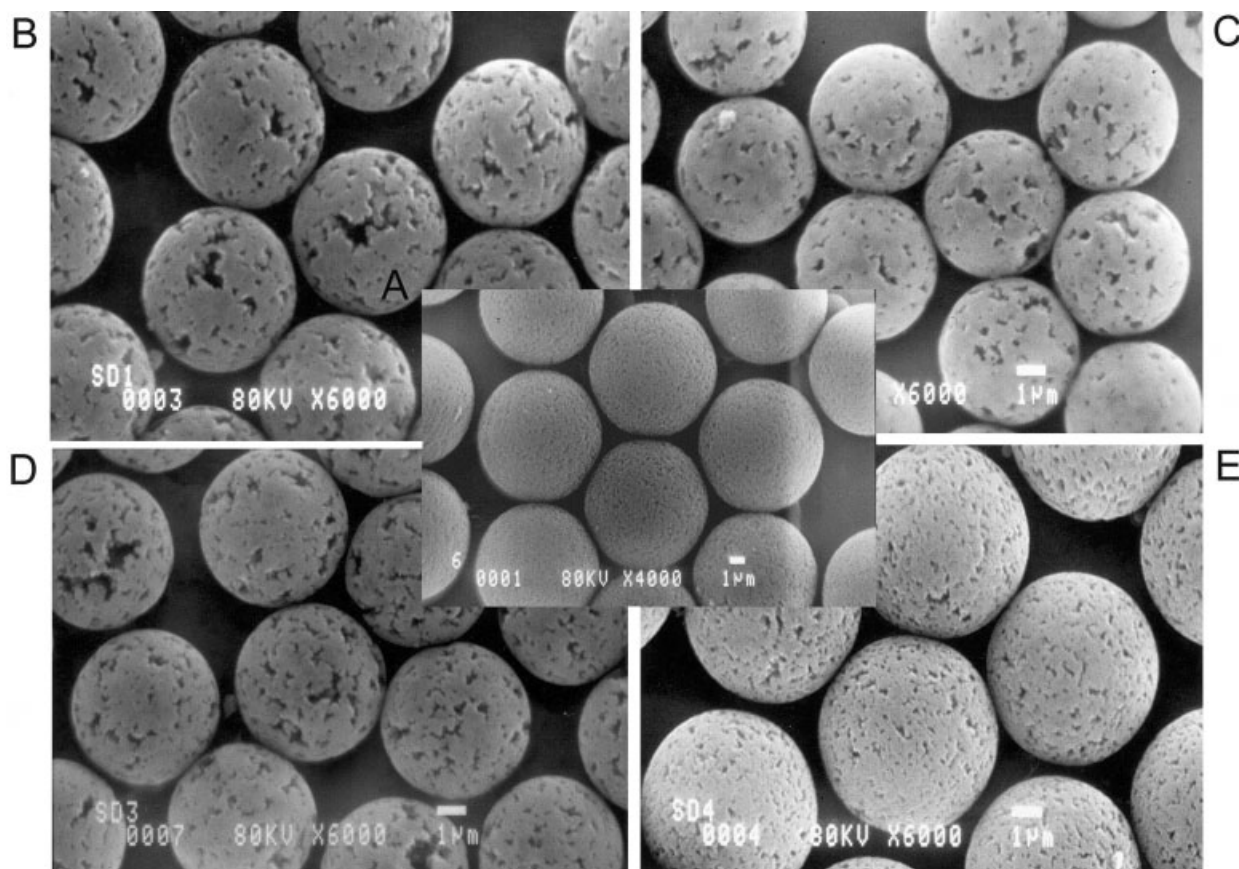


Figure 2 SEM photographs showing the detailed surface morphology of the porous particles. Particle type: (A) SD5, (B) SD1, (C) SD2, (D) SD3, (E) SD4. (Magnification: 4000× for A and 6000× for B–E).

$$h_r = L/N_t d_p \quad (2)$$

RESULTS AND DISCUSSION

Characterization of particles

The properties of seed latices are given in Table I. As seen here, the seed latex 2.1 μm in size had an average molecular weight approximately fourfold higher with respect to the larger one. The CV values, determined by SEM photographs, showed that the larger seed had a slightly wider size distribution. However, the size distribution characteristics of both latices were suitable for the synthesis of porous HPLC packings with narrow size distribution.

In the present study, our aim was to make a further reduction in the size of monodisperse poly(styrene-*co*-divinylbenzene) particles and to achieve higher separation efficiencies in the HPLC column. For this reason, we started with a relatively smaller seed latex (2.1 μm in size) with respect to those used in our previous studies (i.e., 4–5 μm). To generate sufficient porosity within the final particles, we used a porogen mixture including both organic liquid and polymer parts.

The production conditions and the size distribution properties of the monodisperse-porous poly(styrene-

co-divinylbenzene) particles are given in Tables II and III, respectively. The polymerizations performed with the M/SL ratio of 10 mL/g (i.e., the particles encoded as SD1, SD2, and SD3) provided particles approximately 5 μm in size. A representative SEM photograph of the monodisperse-porous poly(styrene-*co*-divinylbenzene) particles encoded as SD3 is given in Figure 1. As seen from the CV values in Table III, no significant difference was observed in the average size with different diluents under identical production conditions. The diluent type did not significantly affect the size distribution. As expected, the use of seed latex 2.1 μm in size, with a higher M/SL ratio (i.e., 16.25 mL/g, SD4), led to the synthesis of particles with larger size (i.e., 6.1 μm) with respect to the particles obtained by the same diluent. On the other hand, the multistage production started with 4.4 μm seed latex resulted in the synthesis of particles 7.8 μm in size (i.e., SD5). In this preparation, M/SL ratio was kept at a relatively lower value (i.e., 4.73 mL/g) for achieving the smallest possible final size and the generation of macropores during the repolymerization stage. Note that the monodisperse particles, in the range of 7–10 μm obtained with the production conditions similar to those used for SD5, exhibited good chromatographic

performance both in SEC and RPLC applications elsewhere.^{30,31}

To provide an idea of the surface morphology (i.e., porosity) of the particles, detailed SEM photographs were taken with a magnification of $\times 6000$. These SEM photographs are presented in Figure 2. As seen here, the sponge-like pore structure was observed for the particles produced with the seed latex $4.4 \mu\text{m}$ in size (Fig. 2A). With respect to the other experiments, the relatively lower average MW of the seed latex is probably responsible for the formation of sponge-like pore structure of SD5 (although a higher amount of seed latex was used for constant monomer volume). All particles synthesized with the seed latex $2.1 \mu\text{m}$ in size using the M/SL ratio of 10 mL/g, had relatively larger pores on their surfaces (i.e., SD1, SD2, and SD3). Extremely larger pores, in the form of craters, were clearly observed on the particles produced by using DBP as the diluent (Fig. 2B). On the other hand, the surface porosity (i.e., the number density of pores on the particle surface) markedly decreased when toluene was the diluent (Fig. 2C). In this sample, crater-like pores were also observed. However, particles with a surface structure including regularly distributed smaller pores were obtained when the toluene-DBP mixture was used as the diluent under the same production conditions (Fig. 2D). This modification also resulted in an increase in the number density of pores on the particle surface. The same diluent system (i.e., toluene-DBP mixture) was also used with a lower amount of seed latex (i.e., with a higher monomer/seed latex ratio) by keeping the other production conditions of SD3 constant. This change led to a significant decrease in the size of the pores on the particle surface (SD4, Fig. 2E). Hence, a sponge-like pore structure was also obtained for particles synthesized with the small seed latex. The surface structures of SD4 and SD5 were reasonably similar because both particles were obtained by using porogen mixtures with relatively lower viscosities.^{23,30}

The SEM photographs, showing the internal structures of these particles, are given in Figure 3. One of the important findings in these photographs is the presence of reasonably large pores in the internal structure of the beads obtained by using pure toluene as the diluent (Fig. 3B). The beads including such large pores should not be considered as a suitable column packing. Relatively smaller pores were observed in the particle interiors obtained by using pure DBP and the DBP-toluene mixture (Figs. 3A and 3C). Hence, these particles were considered as more suitable candidates for HPLC packing.

In the next stage, the particles were slurry packed into stainless steel HPLC columns $150 \times 4.6 \text{ mm}$ in size. The back pressure-flow rate relationships of these columns were obtained by using tetrahydrofuran (THF) as the mobile phase and are given in Figure 4.

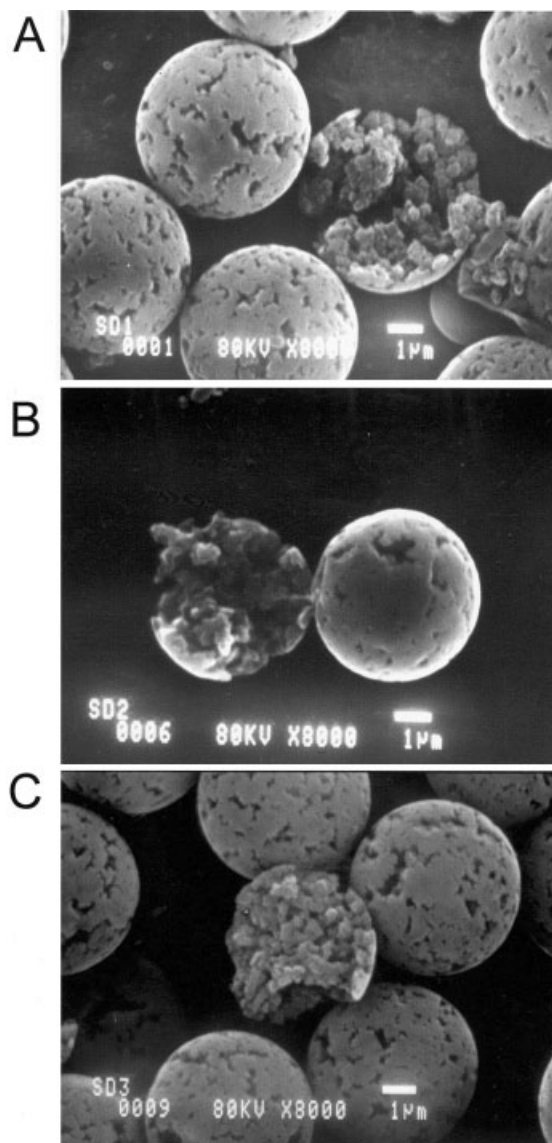


Figure 3 SEM photographs showing the internal structure of the porous particles. Particle type: (A) SD1, (B) SD2, (C) SD3. (Magnification: $8000\times$.)

As seen here, the particles obtained, by using DBP or toluene as the diluent (i.e., SD1 and SD2), provided higher back pressures with respect to the particles produced by using the toluene-DBP mixture (i.e., SD3). The average size of SD3 was nearly the same as those of SD1 and SD2. According to Ergun's equation, the pressure drop in a packed column decreases with increasing column-porosity.³⁵ A chromatographic column with higher porosity is obtained by using particles with higher porosity when the size of the particles in different columns is approximately the same. Hence, lower back pressure, observed with SD3, should be explained by the higher porosity of this material.

In Figure 4, the lowest back pressure was obtained with the largest particles encoded as SD5. Of course,

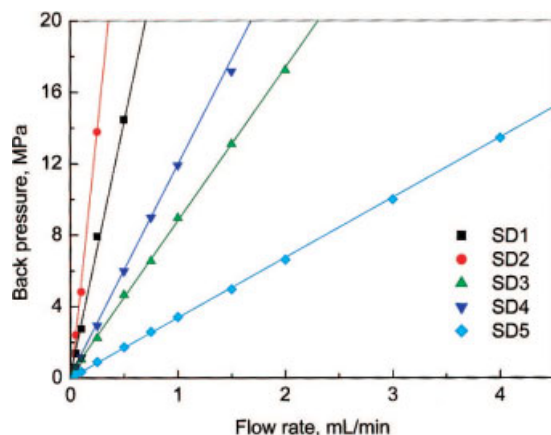


Figure 4 The variation of back pressure with the mobile phase flow rate for columns packed with different particles. Conditions: Column: 150×4.6 mm i.d.; Mobile phase: THF.

an increase in the particle size is a factor reducing the column-back pressure. Based on the collected data, the particles encoded as SD3 and SD4 were selected as possible HPLC packings with relatively smaller size (i.e., $5 \mu\text{m}$) and systematic chromatographic tests were applied to these materials. In these tests, the particles encoded as SD5 were used as a reference since this material was obtained by the same polymerization protocol with a larger size (i.e., $7.8 \mu\text{m}$).

The porosity characteristics of the particles (i.e., SD3, SD4, and SD5), determined by SEC according to the method proposed by Ferreira et al., are given in Table IV.³⁶ For particles obtained by the same seed latex, the particles obtained with higher monomer/seed latex ratio (SD4) had a lower average pore size and higher surface area with respect to the other one (i.e., SD3). This behavior was also observed and explained in our previous studies.^{22,23,30,31} The difference in the average pore size can also be clearly observed in the SEM photographs of SD3 and SD4 (Figs. 2D and 2E). The porosity and pore volume of the reference material (i.e., SD5) were also close to those of smaller particles. The pore size and the surface area of SD5 were between those of SD3 and SD4.

Chromatographic evaluation

The SEC calibration curves of SD3, SD4, and SD5 are given in Figure 5. For the particles obtained with the

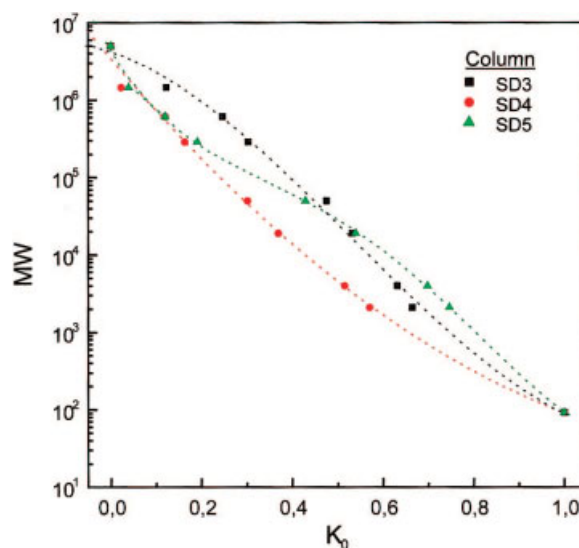


Figure 5 Size-exclusion chromatography calibration curves of the columns packed with small and large particles. Conditions: Column: 150×4.6 mm i.d.; Flow rate: 0.75 mL/min ; UV detection at 254 nm .

small seed latex (i.e., SD3 and SD4), the linearity of the calibration curve was better, particularly in the MW range of 10^3 – 10^6 .

The chromatographic performances of the columns including SD3, SD4, and SD5 were determined comparatively by the isocratic separation of alkylbenzenes. These runs were performed at room temperature, by using acetonitrile (AcN)-water mixture as the mobile phase, with a flow rate of 0.75 mL/min . Here, stainless steel columns 150×4.6 mm in size were used. The chromatographic runs were repeated with different AcN feed concentrations with a UV detector operated at 254 nm . The resolutions, calculated based on the chromatograms obtained with different AcN feed concentrations, are presented in Table V. Where $R(n+1/n)$ values indicate the resolutions between the successive peaks of n and $n+1$. As seen here, the highest resolutions were obtained for SD3 with all AcN concentrations.

The variation of theoretical plate number (TPN) with the AcN feed concentration is given in Figure 6. Again here, the TPNs obtained with SD3 were reasonably higher with respect to those of the reference material encoded as SD5. Note that TPNs up to 40,000

TABLE IV
The Porosity Properties of Particles Determined by SEC

Code	Seed latex size (μm)	Diluent	Average pore size (nm)	Pore volume (mL/g)	Porosity (% v/v)	Specific surface area (m^2/g)
SD3	2.1	Toluene/DBP	95	1.02	56.4	43.3
SD4	2.1	Toluene/DBP	48	1.14	58.6	95.0
SD5	4.4	DBP	62	1.05	55.9	67.8

TABLE V
The Resolutions Calculated Based on the
Chromatograms Obtained with Different AcN Feed
Concentrations

Column: SD3					
AcN % (v/v)	Resolutions				
	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
60	5.11	4.44	5.44	5.97	5.56
70	4.00	3.73	4.37	4.56	4.42
80	3.48	2.95	3.59	3.99	3.74
90	2.48	2.03	2.53	3.02	2.95
100	1.46	1.03	1.37	1.83	1.83
Column: SD4					
AcN % (v/v)	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
80	1.67	1.70	1.97	1.89	1.82
Column: SD5					
AcN % (v/v)	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
60	2.07	2.21	2.83	3.03	3.10
70	2.12	1.97	2.18	2.31	2.33
80	1.83	1.67	1.98	2.24	2.20
90	1.38	1.18	1.49	1.68	1.61
100	0.82	0.55	0.68	0.88	0.95

plates/m could be achieved in the column including SD3 with benzene as the analyte. As a conclusion, the isocratic separation of alkylbenzenes indicated that the chromatographic performance of SD3 was superior with respect to SD5 although their porosity characteristics were not extremely different. Hence, the better chromatographic performance of SD3 should be explained by the lower particle size of this material. It should be noted that TPN values between 6×10^3 and 2.8×10^4 plates/m have been reported using toluene as the analyte in 80:20 AcN:water mixture as the mobile phase, for a column 150×4.6 mm i.d. packed

with the monodisperse poly(styrene-*co*-divinylbenzene) particles 7.5 μ m in size.³⁷

Based on the collected data, the rest of the chromatographic experiments were performed by using only SD3 as the small packing material and SD5 as the reference material with larger size. The effect of the mobile phase flow rate on the chromatographic performance of SD3 was investigated by changing the flow rate between 0.5–2.0 mL/min. The liquid chromatograms of alkylbenzene mixtures obtained with different flow rates are given in Figure 7 for the columns including SD3 and SD5. As seen here, although the flow rate was increased fourfold, the resolution power could be preserved, particularly in the chromatograms obtained with SD3.

The resolution values, calculated based on the chromatograms in Figure 7, are given in Table VI. As seen here, 0.75 mL/min was the flow rate providing the highest resolutions in both columns. However, the resolution values obtained with SD3 were approximately threefold higher than those of SD5 under parallel conditions. In the case of SD3, by increasing the flow rate from 0.5 to 2.0 mL/min (i.e., a fourfold increase), the analysis time decreased from 40 to 10 min by preserving the resolution power. In other words, the analysis period was shortened approximately 75% by preserving the high resolution. However, for the larger particles (i.e., SD5), a clear decrease in the resolution power was observed when increasing the flow rate, particularly from 0.75 to 2.0 mL/min (Table VI). Note that the larger particle size of SD5 allowed use of mobile phase flow rates higher than 2 mL/min. Hence, the flow rate could be increased up to 4 mL/min without exceeding the column filling-pressure. Note that the analysis period achieved with SD5 was approximately 5 min with the flow rate of 3

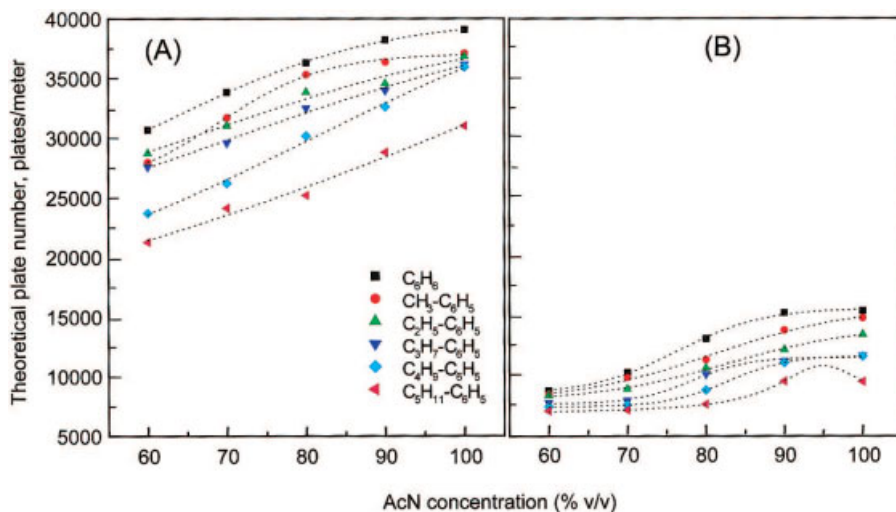


Figure 6 The effect of mobile phase AcN concentration on the theoretical plate number of the columns packed with small and large particles. Column: (A) SD3, (B) SD5.

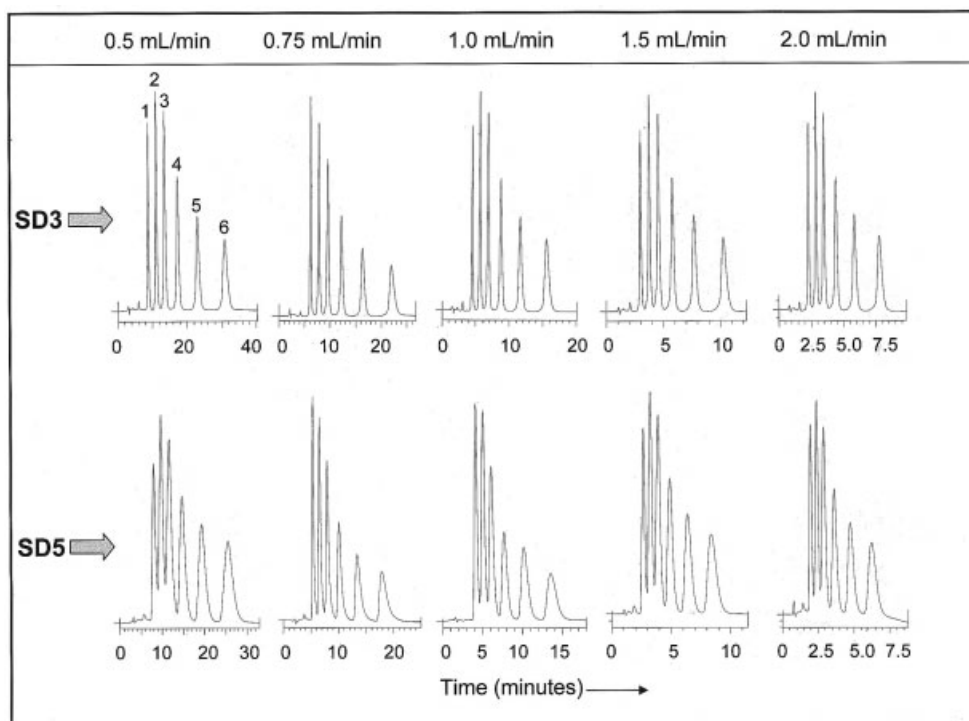


Figure 7 The liquid chromatograms obtained with different mobile phase flow rates for the isocratic separation of alkylbenzenes by the columns packed with relatively small and large particles. Chromatographic conditions: Column: 150×4.6 mm i.d.; AcN concentration: 80% v/v; Mobile phase: AcN-Water; UV detector, 254 nm.

mL/min, at which acceptable resolutions were obtained (Table VI).

The effect of flow rate on column efficiency is shown in Figure 8 by the van Deemter plots comparatively sketched for SD3 and SD5. As seen here, reduced plate

TABLE VI
The Resolutions Calculated Based on the Chromatograms Obtained with Different Mobile Phase Flow Rates

Column: SD3		Resolutions				
Flow rate (mL/min)	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)	
0.50	3.10	2.82	3.44	3.82	3.68	
0.75	3.48	2.95	3.59	3.99	3.74	
1.00	3.20	2.75	3.36	3.89	3.75	
1.50	2.92	2.54	3.17	3.68	3.63	
2.00	3.00	2.60	2.97	3.53	3.47	
Column: SD5		Resolutions				
Flow rate (mL/min)	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)	
0.50	1.05	0.96	1.24	1.64	1.80	
0.75	1.83	1.67	1.98	2.24	2.20	
1.00	1.22	1.17	1.47	1.66	1.71	
1.50	1.20	1.10	1.37	1.59	1.64	
2.00	1.26	1.17	1.41	1.65	1.63	
3.00	1.17	0.96	1.39	1.64	1.67	
4.00	0.96	0.91	1.13	1.42	1.42	

height (h_r) values between 5 and 7 could be achieved for the column packed with the small particles (i.e., SD3). Note that the h_r value of 5 approximately corresponded to 36,300 plates/m. As expected the reference column, packed with the large particles (SD5), provided higher h_r values ranging between 20 and 40. This range was also consistent with the reduced plate height values previously reported for the monodisperse poly(styrene-co-divinylbenzene) particles $7.4 \mu\text{m}$ in size.³⁸

CONCLUSION

Relatively small, monodisperse-porous particles, suitable for chromatographic use, were obtained by "modified seeded polymerization." The use of a toluene-DBP mixture, instead of pure toluene or DBP, as the diluent resulted in significant improvement in the porosity properties of the particles. With the columns including porous poly(styrene-co-divinylbenzene) particles $5.2 \mu\text{m}$ in size, theoretical plate numbers up to 40,000 plates/m could be achieved in the isocratic separation of alkylbenzenes. No significant decrease was observed in the resolution power of the column by fourfold increase of the mobile phase flow-rate. With respect to the currently available polymer based packing materials, significantly higher resolutions and column efficiencies were obtained in RPLC by the packing material synthesized in this study.

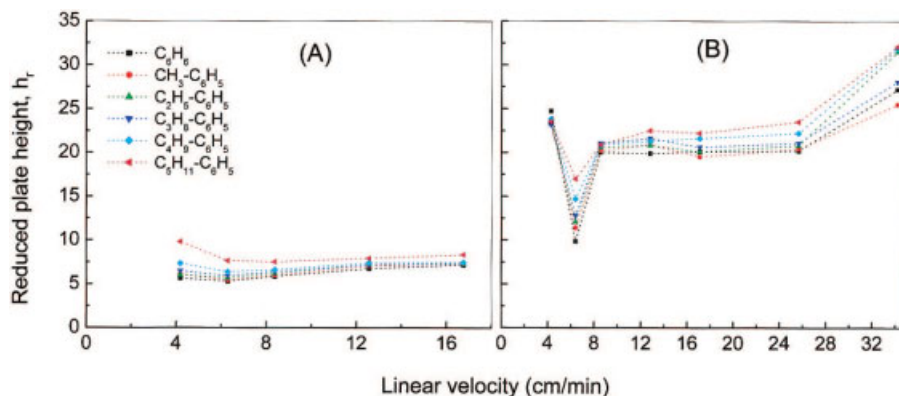


Figure 8 The effect of linear velocity on the efficiency of the columns packed with small and large particles. Column: (A) SD3, (B) SD5. The chromatographic conditions are described in Figure 7.

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