Chromatographic Performance of Monodisperse Macroporous Particles Produced by Modified Seeded Polymerization. II. The Effect of the Diluent/Seed-Latex Ratio

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ABSTRACT: Poly(styrene-*co*-divinylbenzene)-based monodisperse macroporous particles were obtained by a modified seeded polymerization technique. The monodisperse polystyrene particles, obtained by dispersion polymerization in sufficiently large sizes and with suitable average molecular weights, were directly used as the seed latex in the production of macroporous particles. Therefore, the number of swelling and polymerization stages in the multistage production was reduced. In the first stage, the seed particles were swollen with a diluent, dibutyl phthalate (DBP), and then with a monomer phase including styrene, divinylbenzene, and, as an initiator, benzoyl peroxide. The monodisperse macroporous particles were obtained by the repolymerization of the monomer mixture in the seed particles. The particles, having different porosity characteristics, were synthesized through variations in the dibutyl phthalate/seed-latex (DBP/SL) ratio. Selected macroporous particle samples were slurry-packed into stainless steel high-performance liquid chromatography columns (300 mm long × 7.8-mm i.d.). The separation of the protein mixture by these columns in the reversed-phase chromatography (RPC) mode was investigated. Liquid chromatograms with high resolutions were obtained under an acetonitrile/water gradient over a wide range of flow rates (i.e., 0.5–3 mL/min), especially for the particles produced with a monomer/seed-latex (M/SL) ratio of 3.0 mL/g. In the RPC experiments, the particles produced with an M/SL ratio of 3.0 mL/g and DBP/SL ratios of 1.0 and 1.5 mL/g exhibited better chromatographic performance than the other samples. The maximum theoretical plate number was 3500 for the particles produced with the M/SL ratio of 3.0 mL/g and DBP/SL ratio of 1.5 mL/g with albumin as the analyte. The size exclusion chromatography (SEC) calibration curves and the back-pressure/flow-rate relationships of the produced columns were also determined. The particles obtained with an M/SL ratio of 3.0 mL/g and a DBP/SL ratio of 1.5 mL/g exhibited the best performance in SEC applications. J Appl Polym Sci 92: 3685–3696, 2004

Key words: high performance liquid chromatography (HPLC); liquid chromatography; proteins

INTRODUCTION

In high-performance liquid chromatography (HPLC), more regular flow regimes and lower back-pressure are usually obtained in columns including monodisperse packing materials. These advantages provide liquid chromatograms with higher resolutions.[1–4] Various multistage polymerization protocols have been established for the production of monodisperse macroporous particles with different porosity properties and surface chemistries.[1–14] The activated swelling method was one of the first methods proposed for the synthesis of monodisperse compact/macroporous particles in the range of 1–20 μm.[1,5,6] El-Aasser and coworkers[2,7] established a production method for uniform macroporous particles, 10 μm in diameter, via seeded emulsion polymerization.[2,7] A staged template polymerization for the synthesis of monodisperse porous poly(styrene-*co*-divinylbenzene) [poly(S-DVB)] particles, 7.4 μm in size, was developed by Frechet and coworkers.[3,8] The Shirasu porous glass technique was recently developed for the synthesis of monodisperse macroporous particles in the size range of 2.5–60 μm.[10,11]

Recently, we proposed a modified multistage polymerization protocol for the synthesis of monodisperse macroporous particles potentially suitable as chromatographic packing materials in HPLC.[13,14] Although the proposed method was based on the principles established by Ugelstad et al.[1] and Galia et al.,[3] we introduced some modifications to obtain macroporous particles with improved monodispersity.[13,14] In the first stage of our production method, we established a dispersion polymerization protocol for the

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TABLE I
Production Conditions of the Monodisperse Macroporous Particles

Code	Seed latex (g)	DBP (mL)	Styrene (mL)	DVB (mL)	BPO (g)
TG10	2.50	2.50	2.50	7.50	0.375
TG6	2.50	3.75	2.50	7.50	0.375
TG11	2.50	5.00	2.50	7.50	0.375
TG12	3.33	3.33	2.50	7.50	0.375
TG8	3.33	5.00	2.50	7.50	0.375
TG13	3.33	6.66	2.50	7.50	0.375
TG14	4.25	4.25	2.50	7.50	0.375
TG9	4.25	6.38	2.50	7.50	0.375
TG15	4.25	8.50	2.50	7.50	0.375

synthesis of the seed latex, including sufficiently large, monodisperse particles with a suitable average molecular weight.[13–15] In the multistage protocol, this latex was used as a part of the porogen mixture without any additional swelling stage for the adjustment of the molecular weight and particle size of the starting material. After the swelling of the seed latex by an organic diluent and a monomer mixture, the final macroporous particles were obtained in a highly monodisperse form by a repolymerization stage. Therefore, the number of emulsification and swelling stages in the multistage production protocol was reduced.[13] The multistage polymerization protocol proposed for particles based on styrene divinylbenzene was also applied to the production of monodisperse macroporous particles with different surface chemistries. Monodisperse macroporous particles with polarities were also obtained through the addition of different functional acrylic monomers (i.e., 2-hydroxyethylmethacrylate, methacrylic acid, glycidyl methacrylate, and 4-vinylphenylboronic acid) to the polymerization recipe.[16–23]

Various chromatography studies were performed to determine the chromatographic performance of monodisperse macroporous particles, particularly by Frechet and coworkers.[24–30] The chromatographic performance of monodisperse macroporous particles, based on styrene divinylbenzene and produced with a staged shape template polymerization, was tested with both size exclusion chromatography (SEC) and reversed-phase chromatography (RPC).[3,8] Monodisperse beads based on hydrolyzed macroporous poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) were synthesized and used as size exclusion HPLC packing.[24] The porosity properties of monodisperse poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads were also controlled with chain-transfer agents in the multistage seeded polymerization.[25] Novel separation media for HPLC were prepared from monodisperse glycidyl methacrylate-*co*-ethylene dimethacrylate beads with a pore-size-specific functionalization process.[26,27] The monodisperse macroporous beads produced by the staged shape template copolymerization of 2,3-epoxypropylvinylbenzylether with divinylbenzene were examined as packing materials in the RPC of alkylbenzenes and proteins.[28] Recently, polar stationary phases based on monodisperse macroporous poly(2,3-dihydroxypropyl methacrylate-*co*-ethylene dimethacrylate) beads were developed for normal-phase HPLC applications.[29,30]

As a part of the systematic testing of the chromatographic performance of the monodisperse macroporous particles obtained by our multistage polymerization protocol, this study was designed.[23] For this purpose, styrene divinylbenzene copolymer particles in a suitable size range were synthesized through variations in the diluent/seed-latex ratio. The produced particles were used as column-packing materials in HPLC, and their chromatographic performance was investigated in both SEC and RPC modes.

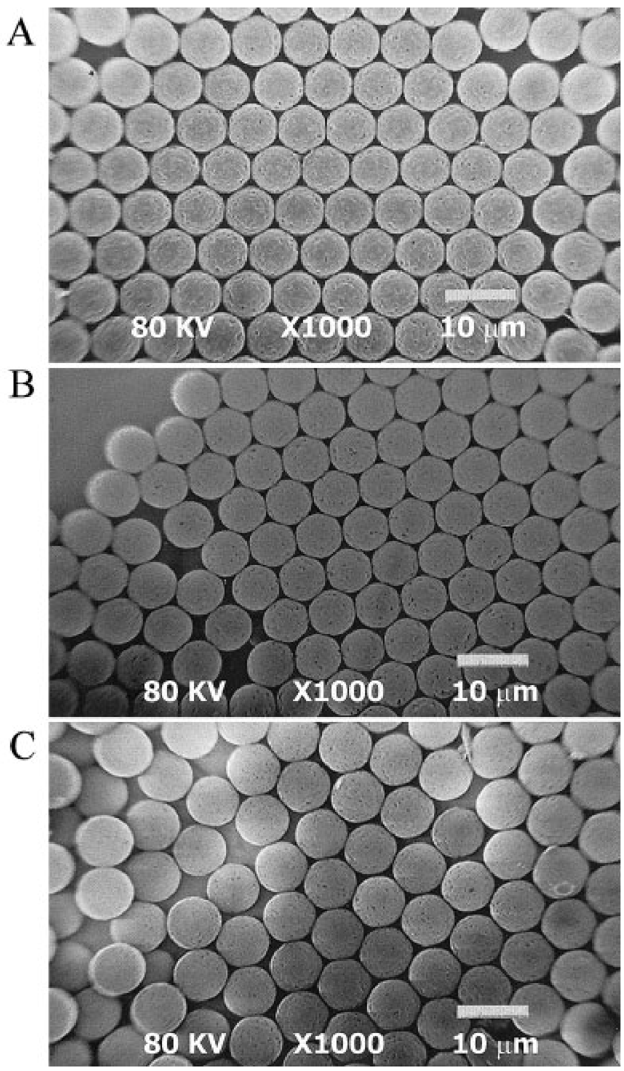

Figure 1 SEM photographs exemplifying the monodispersity of poly(S-DVB) particles produced with different DBP/SL ratios (M/SL ratio = 3.00 mL/g, original magnification = 1000×): (A) 1.0, (B) 1.5, and (C) 2.0 mL/g.

TABLE II
Variation of the Average Size (D_n) and Size Distribution Properties of Monodisperse Macroporous Particles Obtained with Different DBP/SL Ratios

Code	M/SL (mL/g)	DBP/SL (mL/g)	D_n (μm)	CV (%)
TG10	4.00	1.0	7.11	2.53
TG6	4.00	1.5	7.13	3.61
TG11	4.00	2.0	7.91	0.88
TG12	3.00	1.0	6.69	2.46
TG8	3.00	1.5	7.12	1.59
TG13	3.00	2.0	7.94	1.30
TG14	2.35	1.0	7.01	2.79
TG9	2.35	1.5	7.53	2.00
TG15	2.35	2.0	6.29	1.88

EXPERIMENTAL

Materials

Styrene (Yarpet AS, Kocaeli, Turkey) was distilled *in vacuo*. Dibutyl phthalate (DBP; Aldrich Chemical Co., Milwaukee, WI) was selected as the diluent for the preparation of the monodisperse macroporous particles. Divinylbenzene (including 55% *para*- and *meta*-divinylbenzene isomers; Aldrich Chemical) was extracted with a 5% (w/w) NaOH solution. Sodium lauryl sulfate (SDS; Sigma Chemical Co., St. Louis, MO) was used as an emulsifier in the preparation of an aqueous emulsion medium for the swelling of polystyrene (PS) seed particles. Benzoyl peroxide (BPO; Aldrich Chemical) was used as an oil-soluble initiator in the repolymerization stage. Acetonitrile (HPLC-grade; Aldrich Chemical) was used as the mobile phase in RPC. Bovine serum albumin (BSA; catalog number A-2153, fraction V, molecular weight = 67,000), lysozyme (catalog number L-6876, molecular weight = 17,000), ribonuclease A (catalog number R-5503, molecular weight = 13,200), and cytochrome C (catalog number C-2037, molecular weight = 14,200) were selected as the protein standards and were purchased from Sigma Chemical. Distilled and deionized water was used in all the polymerizations.

Synthesis of the monodisperse macroporous particles

A multistage seeded polymerization protocol was used for the synthesis of the monodisperse macro-

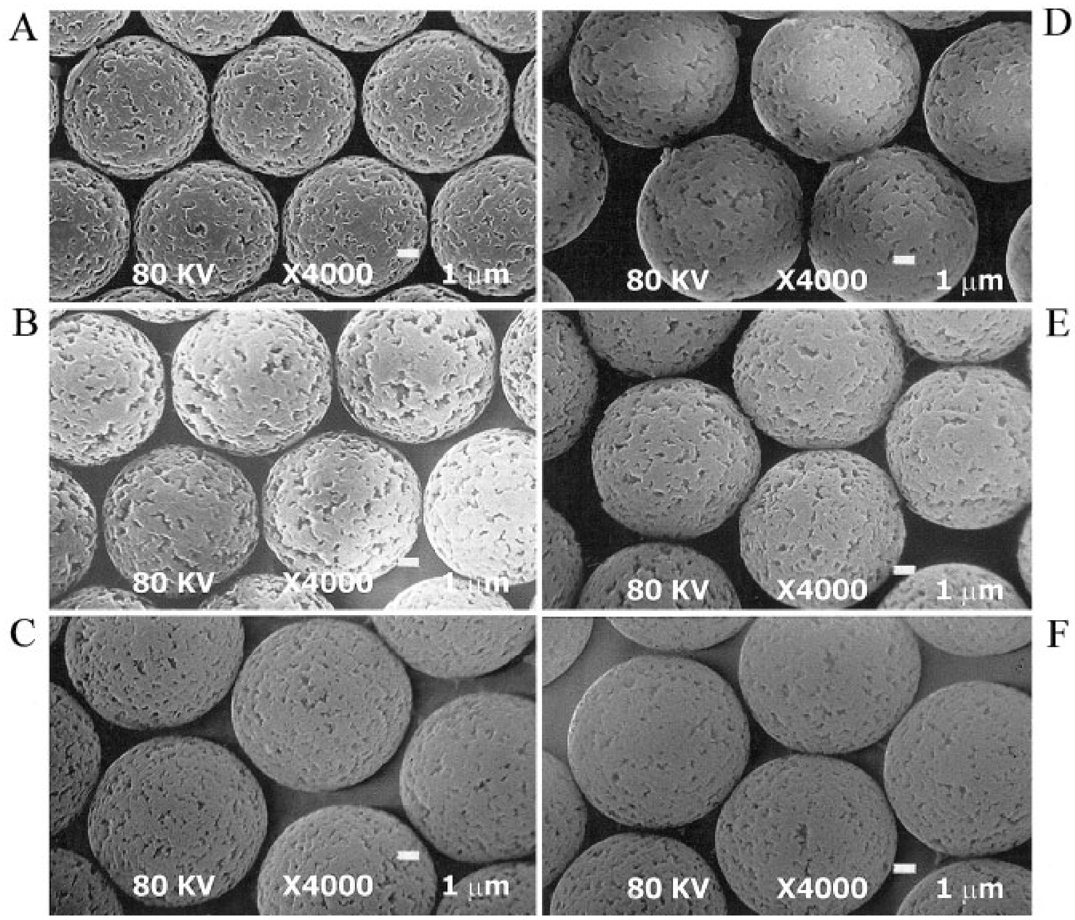

Figure 2 SEM photographs showing the detailed surface morphology of the particles produced with M/SL ratios of 3.0 and 3.5 mL/g (original magnification = 4000×). The M/SL and DBP/SL ratios were (A) 3.0 and 1.0 mL/g, (B) 3.0 and 1.5 mL/g, (C) 3.0 and 2.0 mL/g, (D) 3.5 and 1.0 mL/g, (E) 3.5 and 1.5 mL/g, and (F) 3.5 and 2.0 mL/g.

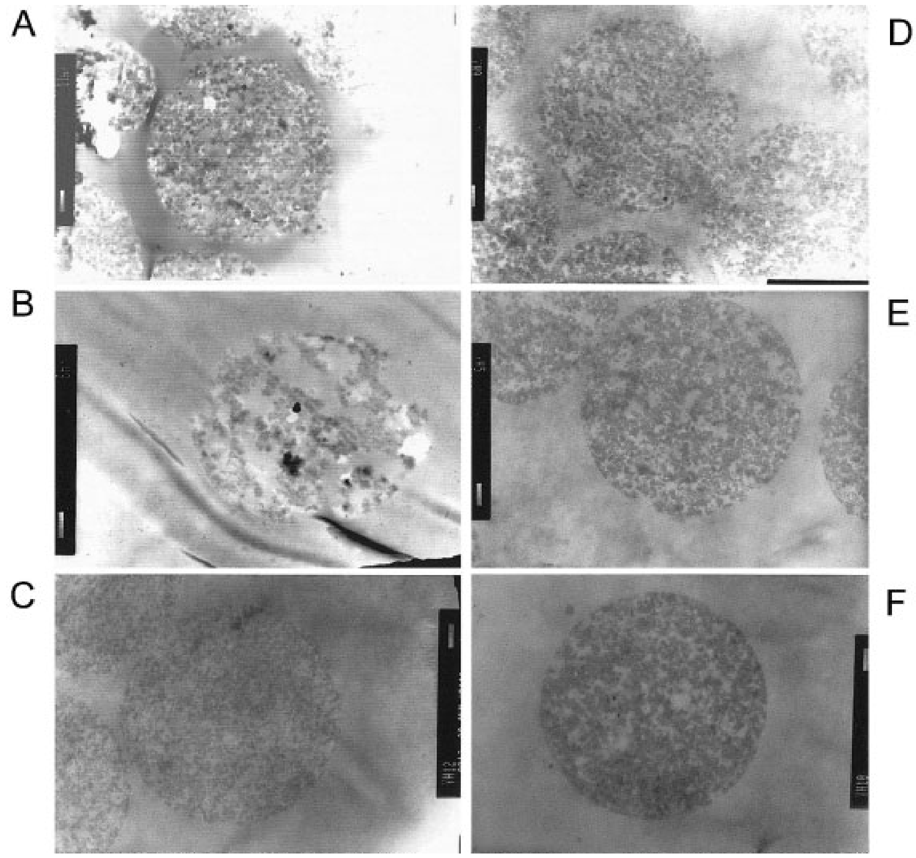

Figure 3 TEM photographs of thin cross sections of the particles produced with M/SL ratios of 3.0 and 3.5 mL/g (original magnification = 5000×). The M/SL and DBP/SL ratios were (A) 3.0 and 1.0 mL/g, (B) 3.0 and 1.5 mL/g, (C) 3.0 and 2.0 mL/g, (D) 3.5 and 1.0 mL/g, (E) 3.5 and 1.5 mL/g, and (F) 3.5 and 2.0 mL/g.

porous latex particles. The monodisperse PS particles, 4.8 μm in size, were prepared by dispersion polymerization and were used as the seed latex in this protocol.[23] A typical procedure for the multistage seeded polymerization was as follows. The diluent DBP (5.0 mL) was emulsified in an aqueous medium (100 mL) including 0.25% (w/w) SDS as the emulsifier. The mixture of DBP and the aqueous SDS solution was sonicated for 45 min in an ultrasonic bath (Bransonic 200, Providence, RI) for complete emulsification. Then, 20 mL of the latex dispersion, including 3.33 g of PS seed particles, was added into an emulsion prepared previously. For the absorption of DBP by the PS seed particles, the new dispersion medium was stirred magnetically (400 rpm) at room temperature for 24 h.

In the next stage, a monomer phase consisting of styrene (2.5 mL), DVB (7.5 mL), and BPO (0.375 g) was emulsified in an aqueous medium (120 mL) including 0.25% (w/w) SDS. This emulsion was added to the dispersion containing DBP-swollen seed particles. The new emulsion was stirred at room temperature for 24 h at 400 rpm for the absorption of the monomer phase by the DBP-swollen seed particles.

Finally, an aqueous poly(vinyl alcohol) solution (6 mL, 20% w/w) was added to the resulting dispersion and purged with nitrogen for 5 min. The repolymerization of the monomer phase in the swollen seed particles was conducted at 70°C at a 120 cpm shaking rate for 24 h. An extensive washing-and-extraction procedure was performed after the production. This protocol has been defined in detail elsewhere.[23]

Characterization of the particles

The size distribution properties and surface morphology of the macroporous particles were investigated with a scanning electron microscope (JEM1200EX, JEOL, Tokyo, Japan). The internal structure of the particles was evaluated with a transmission electron microscope (JEM 1200 EX, JEOL). The characterization procedure has been reported in detail elsewhere.[23] The porosity properties of the particles {i.e., pore size distribution, average pore size (Å), pore volume [V_p (mL/g)], and porosity [P (% v/v)]} were calculated with the retention volumes of the standards in the SEC study according to the method used by Ferreira et al.[31] V_p was calculated on the basis of the retention volume difference between toluene and the PS standard with a molecular weight of 5,000,000.

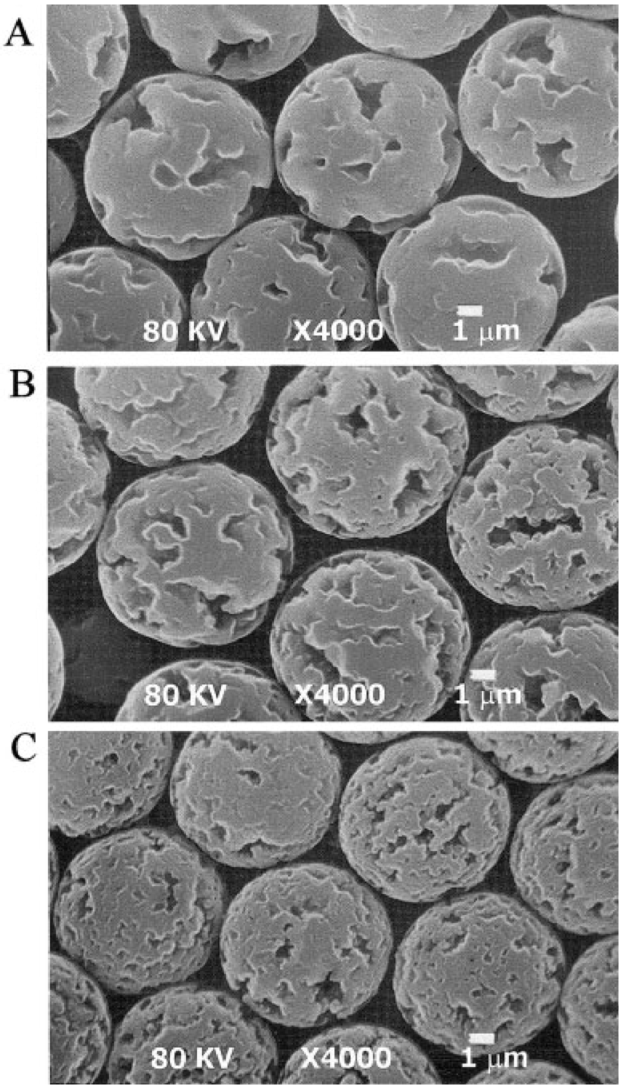

Figure 4 SEM photographs showing the detailed surface morphology of the particles produced with the M/SL ratio of 2.35 mL/g (original magnification = 4000×) and different DBP/SL ratios: (A) 1.0, (B) 1.5, and (C) 2.0 mL/g.

Chromatographic study

The monodisperse macroporous particles were slurry-packed into stainless steel HPLC columns (300 mm long × 7.8-mm i.d.) under a pressure of 130 atm. BSA, lysozyme, ribonuclease A, and cytochrome C were selected as the protein standards. The chromatographic tests were performed in a liquid chromatograph (Shimadzu, Tokyo, Japan) equipped with a ternary gradient pump (LC-10 ADVP) and a UV detector (SPD-10 AVVP). The analysis of the protein mixture was performed under an acetonitrile/water gradient including trifluoroacetic acid (TFA; Aldrich Chemical) in the mobile phase at a concentration of 0.15% (v/v). The chromatograms were recorded at 280 nm with different flow rates of 0.5–3.0 mL/min. The theoretical plate number (TPN) and the reduced plate height (RPH) were calculated from the liquid chromatograms of BSA recorded in the isocratic mode with an acetonitrile/water mixture (25:75) at different flow rates of 0.5–3.0 mL/min.[23]

SEC was performed in tetrahydrofuran (THF) with stainless steel columns (300 mm long × 7.8-mm i.d.). During the SEC experiments, the mobile-phase flow rate was adjusted to 1.0 mL/min, and the PS standards (molecular weight = 5,000,000, 1,460,000, 692,000, 288,000, 92,000, 50,000, 19,200, 4,000, and 2,100) and toluene (molecular weight = 92) were injected into the column. The chromatograms were obtained with an SPD-10 AVVP UV detector at 254 nm.

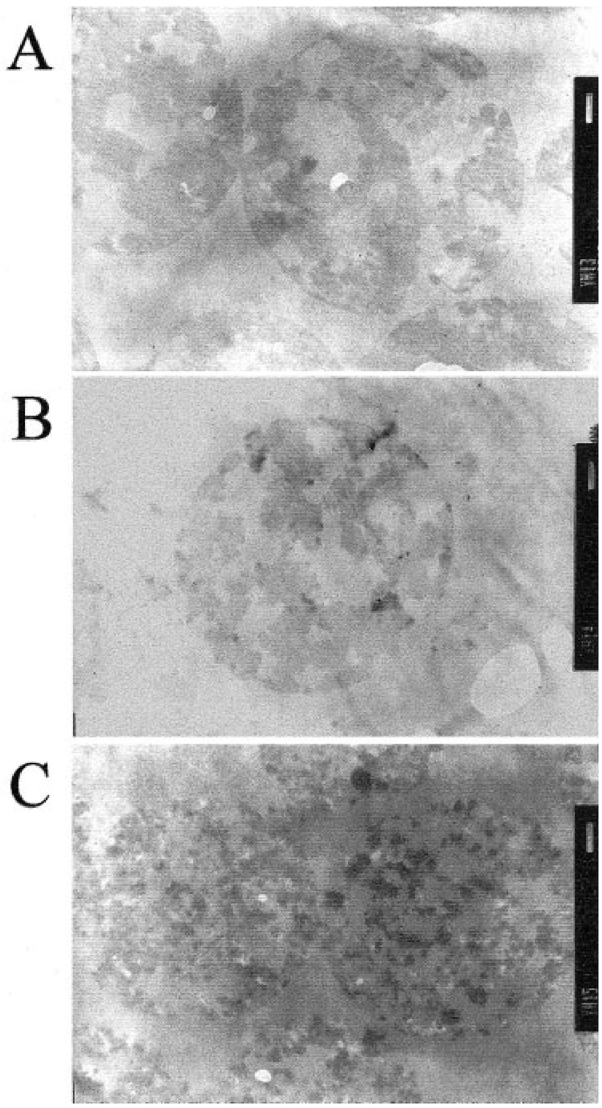

Figure 5 TEM photographs of thin cross sections of the particles produced with the M/SL ratio of 2.35 mL/g (original magnification = 5000×) and different DBP/SL ratios: (A) 1.0, (B) 1.5, and (C) 2.0 mL/g.

TABLE III
Gradient Conditions Used for the Separation of Proteins by RPC on a Column Packed with Particles Obtained with Different DBP/SL Ratios

Column code	M/SL (mL/g)	DBP/SL (mL/g)	Initial A (%)	Final A (%)	Time (min)
TG12	3.00	1.0	30	50	30
TG8	3.00	1.5	30	60	30
TG13	3.00	2.0	30	60	35
TG9	2.35	1.5	30	60	40
TG15	2.35	2.0	30	55	30

Conditions: Column = 30 × 7.8-mm i.d.: mobile phase A = 5% water in acetonitrile and 0.15% TFA, B = water and 0.15% TFA; flow rate = 1 mL/min; UV detection at 280 nm.

RESULTS AND DISCUSSION

Characterization of the particles

The poly(S-DVB) particles were obtained through changes in the dibutyl phthalate/seed-latex (DBP/SL) ratio at selected monomer/seed-latex (M/SL) ratios. The production conditions are given in Table I. Scanning electron microscopy (SEM) photographs exemplifying the monodispersity of the poly(S-DVB) particles are given in Figure 1. The particle size and size distribution properties of the particles are listed in Table II. All the coefficient of variation (CV) values were less than 5%, and so the particles had a reasonably narrow size distribution. With a constant M/SL ratio, the average particle size slightly increased with an increasing DBP/SL ratio. The size distribution became narrower, particularly at high DBP/SL ratios

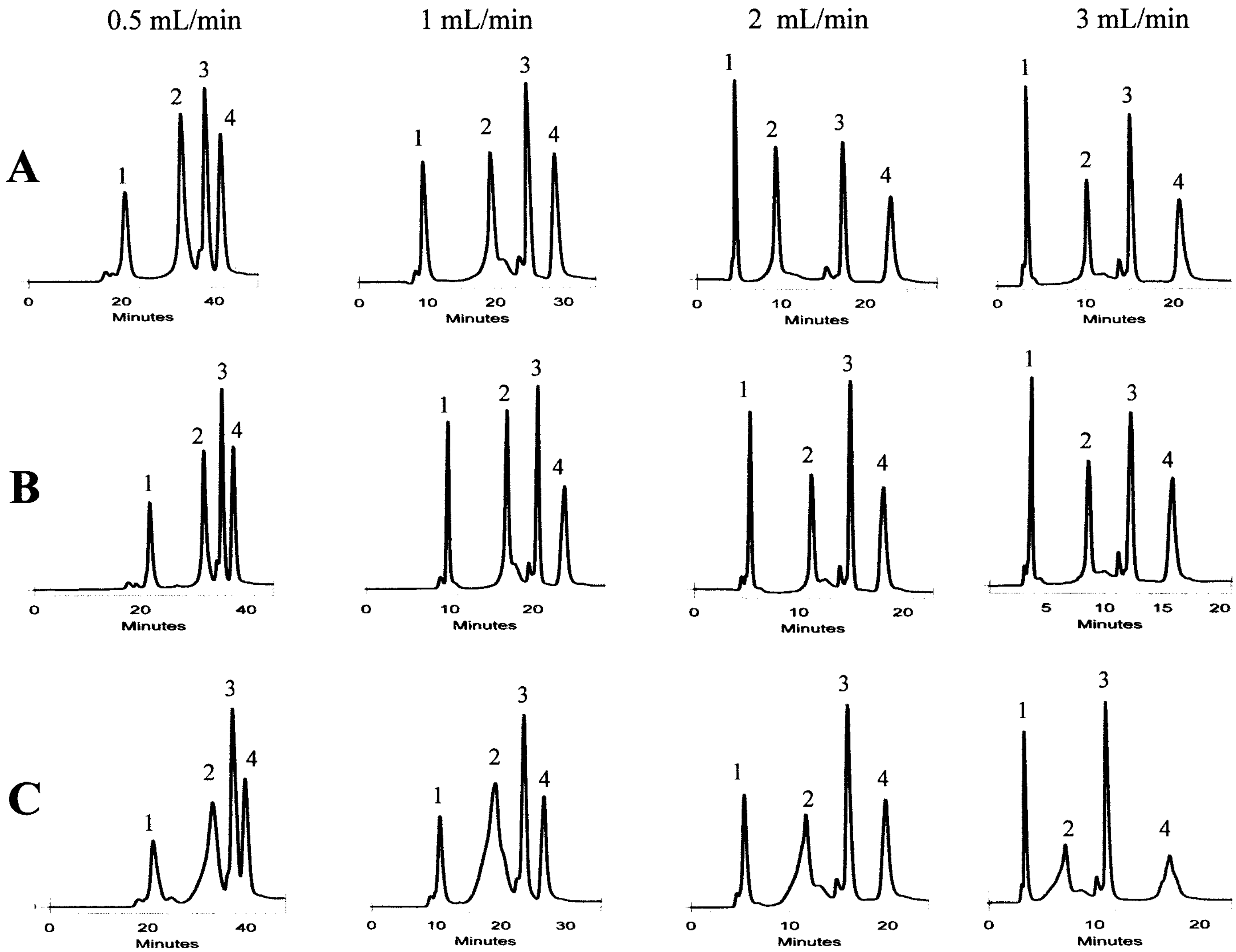

Figure 6 Separation of the protein mixture by RPC with different mobile-phase flow rates on a column packed with particles produced with different DBP/SL ratios (linear gradient of acetonitrile in water; column, 300 mm long × 7.8-mm i.d.; variable flow rate = 0.5–3.0 mL/min; UV detection at 280 nm). The M/SL ratio was 3.0 mL/g, and the DBP/SL ratios were (A) 1.0, (B) 1.5, and (C) 2.0 mL/g. The elution order was (1) ribonuclease A, (2) cytochrome C, (3) lysozyme, and (4) albumin.

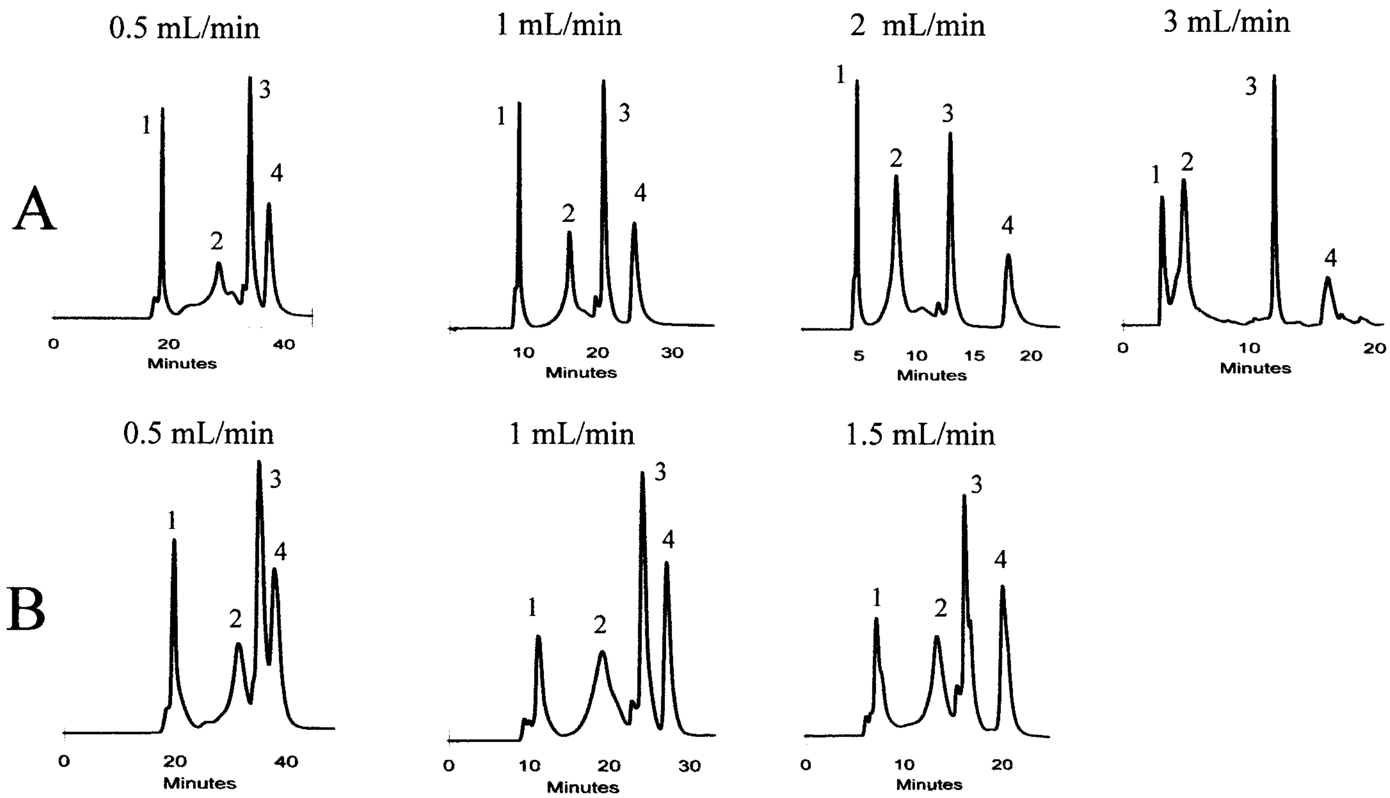

Figure 7 Separation of the protein mixture by RPC with different mobile-phase flow rates on a column packed with particles produced with different DBP/SL ratios (linear gradient of acetonitrile in water; column, 300 mm long × 7.8-mm i.d.; variable flow rate = 0.5–3.0 mL/min; UV detection at 280 nm). The M/SL ratio was 2.35 mL/g, and the DBP/SL ratios were (A) 1.5 and (B) 2.0 mL/g. The elution order was (1) ribonuclease A, (2) cytochrome C, (3) lysozyme, and (4) albumin.

(e.g., 2.0 mL/g). The monomer phase should be absorbed more easily by seed particles swollen with larger amounts of DBP. The possibility of an equal distribution of the monomer phase among the seed particles is probably stronger when the monomer absorption is easier. SEM photographs showing the detailed surface morphology of the particles produced with M/SL ratios of 3.0 and 3.5 mL/g are given in Figure 2. A spongelike structure, including relatively smaller pores homogeneously distributed throughout the particle surface, can be observed. Transmission electron microscopy (TEM) photographs of thin cross sections of the same particles are given in Figure 3. A spongelike pore structure very similar to that of the surface can be observed for the particle interior. SEM photographs showing the detailed surface morphology of the particles produced with the M/SL ratio of 2.35 mL/g are given in Figure 4. The pore structure on the particle surface was reasonably different from that shown in Figure 2. Instead of a spongelike structure, a craterlike porosity, including relatively larger pores irregularly distributed to the particle surface, can be observed for the surfaces of the particles obtained with different DBP/SL ratios at the M/SL ratio of 2.35 mL/g. TEM photographs of thin cross sections of the particles produced with the M/SL ratio of 2.35 mL/g are included in Figure 5. The pore structure in the particle interior was similar to that of the surface shown in Figure 4. In our method, the porogen mixture consists of DBP and linear PS coming from the seed particles. An increase in the DBP/SL ratio with a constant amount of the seed latex involves an increase in the porosity of the final particles. Cheng and coworkers[2,7] proposed a mechanism for the pore formation process during the synthesis of monodisperse macroporous particles from PS seed latex. According to this mechanism, highly crosslinked gel microspheres are generated in the first stage of the pore formation process through the phase separation occurring between the porogen mixture and crosslinked polymer. The adhesion and combination of the crosslinked microspheres result in the formation of aggregates in the forming particle structure. In the final stage, the fixation and binding of these agglomerates occur, and the voids between the agglomerates are filled with the linear polymer and the diluent.[2,7] As shown in Figures 2–5, the mean pore size decreased with an increasing DBP/SL ratio. This behavior was valid for the pores both on the particle surface and in the particle interior and was particularly clear for the particles produced with the M/SL ratio of 2.35 mL/g. This finding, also observed in our previous studies,

TABLE IV
Resolution Values of the Chromatograms Given in Figures 6 and 7

Resolution parameters	Flow rate (mL/min)			
TG12, DBP/SL = 1.0 mL/g	0.5	1	2	3
R(II/I)	3.03	4.00	3.32	5.81
R(III/II)	1.19	2.22	4.74	3.89
R(IV/III)	1.19	2.05	3.65	3.14
TG8, DBP/SL = 1.5 mL/g	0.5	1	2	3
R(II/I)	3.92	6.11	5.81	5.53
R(III/II)	1.23	3.02	4.04	3.50
R(IV/III)	1.13	2.55	3.05	3.17
TG13, DBP/SL = 2.0 mL/g	0.5	1	2	3
R(II/I)	2.33	2.04	2.68	2.05
R(III/II)	1.18	1.15	2.08	2.42
R(IV/III)	0.69	1.22	1.80	3.19
TG9, DBP/SL = 1.5 mL/g	0.5	1	2	3
R(II/I)	3.21	3.56	3.56	1.00
R(III/II)	1.08	1.91	2.79	3.76
R(IV/III)	1.23	2.08	2.96	3.26
TG15, DBP/SL = 2.0 mL/g	0.5	1.0	1.5	
R(II/I)	2.74	1.81	2.55	
R(III/II)	0.75	1.14	1.52	
R(IV/III)	0.51	1.42	1.91	

can be explained by the formation of smaller aggregates with an increasing DBP content in the porogen mixture.[13,18,21,22] Therefore, the voids (i.e., pores) between the fixed aggregates in the forming particle structure should be smaller.

Chromatographic evaluation

An evaluation of SEM and TEM photographs given in Figures 2–5 showed that the surface and internal morphologies (i.e., the pore structures) of the particles produced with the M/SL ratios of 3.0 and 4.0 mL/g were reasonably similar. As mentioned previously, the particles in both series had spongelike porosity for all DBP/SL ratios tried. However, the pore structure of the particles produced with the M/SL ratio of 2.35 mL/g was reasonably different, and these particles exhibited craterlike porosity (i.e., extremely larger pores) with all DBP/SL ratios.

To observe the chromatographic behavior of particles with different pore structures, we selected the particles produced with the M/SL ratios of 2.35 and 3.0 mL/g as packing materials for HPLC. The columns (300 mm long × 7.8-mm i.d.), packed with the particles obtained with different DBP/SL ratios, were tested in the reversed-phase mode. In the chromatographic experiments, the separation of a protein mixture, including four proteins with different molecular weights and hydrophobicities, was investigated under an acetonitrile/water gradient at room temperature. To determine the appropriate gradient conditions for each column, we performed a set of preliminary chromatographic experiments, and the conditions providing the highest resolution values were determined at a mobile-phase flow rate of 1 mL/min. Then, each column (i.e., each packing material obtained with certain M/SL and DBP/SL ratios) was tested at different flow rates under the gradient conditions providing the best separation at 1.0 mL/min. The columns and the gradient conditions are given in Table III. The concentration of mobile phase A (95% acetonitrile and 5% water) was initially fixed at 30% (v/v) and increased to 50

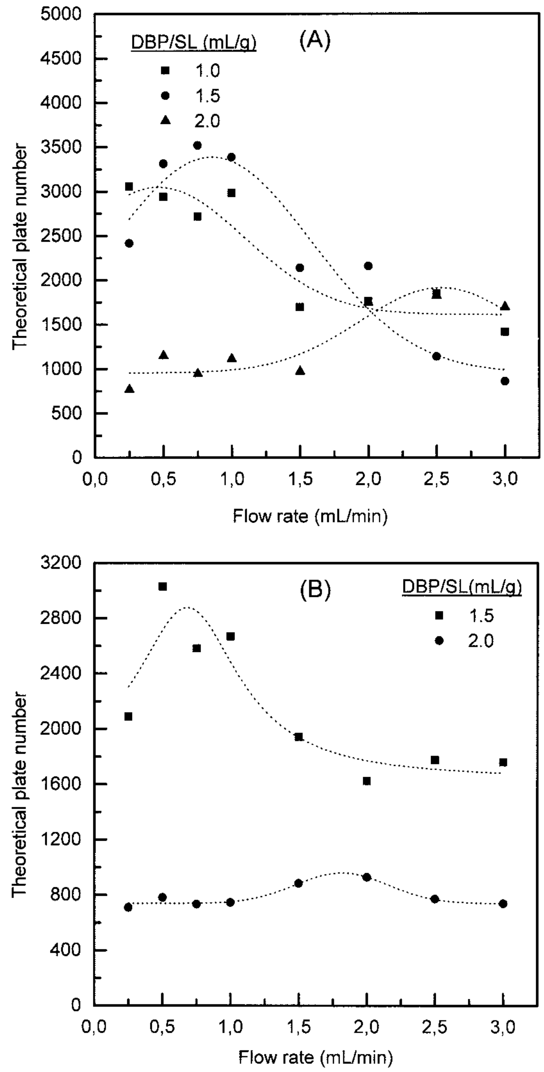

Figure 8 Effect of the flow rate on the TPN of a column packed with particles obtained with different DBP/SL ratios (column, 300 mm long × 7.8-mm i.d.; mobile phase = 75:25 acetonitrile/water with 0.15% TFA; UV detection at 280 nm; analyte = albumin). The M/SL ratios were (A) 3.0 and (B) 2.35 mL/g.

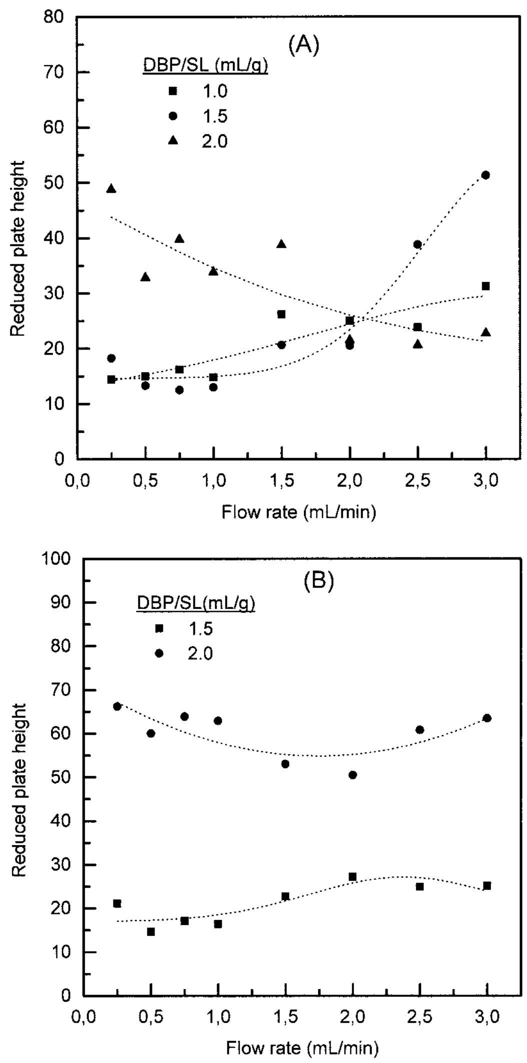

Figure 9 Effect of the flow rate on the RPH of a column packed with particles obtained with different DBP/SL ratios (column, 300 mm long × 7.8-mm i.d.; mobile phase = 75:25 acetonitrile/water with 0.15% TFA; UV detection at 280 nm; analyte = albumin). The M/SL ratios were (A) 3.0 and (B) 2.35 mL/g.

or 60% (v/v) in a period of 30–40 min. In other words, the slope of the gradient was equal or close to 1.0%/min for the columns tested. The liquid chromatograms recorded at different flow rates for the columns packed with the particles produced with the M/SL ratios of 3.0 and 2.35 mL/g are given in Figures 6 and 7, respectively. The resolution values calculated on the basis of these chromatograms are presented in Table IV.

As shown in Figures 6 and 7 and Table IV, the column materials obtained with the M/SL ratio of 3.0 mL/g exhibited better chromatographic performance than those produced with the M/SL ratio of 2.35 mL/g. Among the materials produced with the M/SL ratio of 3.0 mL/g, the particles synthesized with the DBP/SL ratios of 1.0 and 1.5 mL/g provided satisfactorily high-resolution values. The particles synthesized with the DBP/SL ratio of 2.0 mL/g showed lower separation performance at both M/SL ratios. However, the resolution exhibited a maximum at intermediate flow rates (mostly at 2 mL/min; Table IV). The increase in the resolution may be attributed to the increasing effect of intraparticular convective transport with the increasing flow rate.[3,8,23] The decrease observed after the maximum point can probably be explained by the insufficient (i.e., short) residence time of the analyte in the column due to the high flow rate. The column containing the particles produced with the M/SL ratios of 2.35 and 2.0 mL/g could not be examined at a flow rate of 3.0 mL/min because the column pressure at this flow rate was close to the pressure used in the packing of the column material.

The effect of the flow rate on TPN is given in Figure 8. For all columns, TPN exhibited a maximum point.

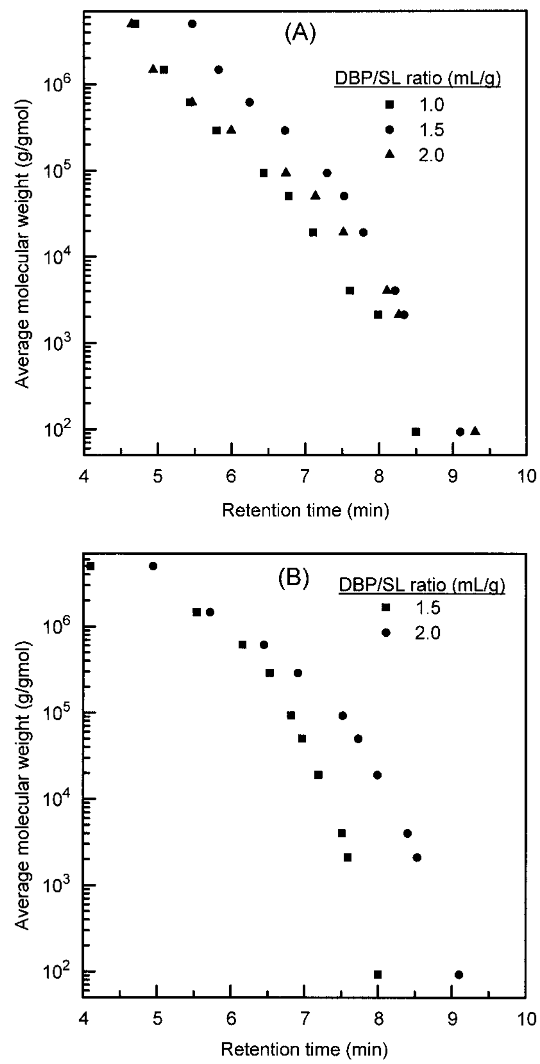

Figure 10 SEC calibration curves for PS standards in THF. The columns were packed with particles obtained with different DBP/SL ratios (column, 300 mm long × 7.8-mm i.d.; flow rate = 1.0 mL/min; UV detection at 254 nm). The M/SL ratios were (A) 3.0 and (B) 2.35 mL/g.

TABLE V
Porosity Properties of the Particles Calculated with SEC

Column code	M/SL (mL/g)	DBP/SL (mL/g)	D_n (nm)	V_p (mL/g)	P (% v/v)
TG12	3.00	1.0	93	0.54	39
TG8	3.00	1.5	98	0.58	41
TG13	3.00	2.0	83	0.77	48
TG9	2.35	1.5	198	0.51	35
TG15	2.35	2.0	140	0.66	44

D_n = average particle size.

The maximum TPN value was observed at relatively lower flow rates (i.e., between 0.5 and 1.0 mL/min) for the particles produced with the DBP/SL ratio of 1.0 or 1.5 mL/g [Fig. 8(A,B)]. For these particles, TPN markedly decreased with an increasing flow rate. For both M/SL ratios, the particles produced with the DBP/SL ratio of 2.0 mL/g exhibited a maximum TPN value at a higher flow rate (i.e., 2.0 mL/min). Among the materials tested, the particles obtained with the M/SL ratio of 3.0 mL/g and the DBP/SL ratio of 1.5 mL/g provided the highest TPNs in the flow-rate range of 0.5–2.0 mL/min [Fig. 8(A)].

The variation of RPH with the flow rate is shown for the particles obtained with both M/SL ratios in Figure 9. The efficiency of a column packed with a conventional separation medium decreases linearly with an increasing flow rate.[8] Contrary to this tendency, the RPH of our columns exhibited a slight increase or remained roughly constant with an increasing flow rate, in most cases. This result can be explained by the increasing importance of intraparticular convection with an increasing flow rate for particles with relatively larger pores. This case probably prevented a reduction in the column efficiency, particularly for the particles with large macropores (i.e., the ones obtained with the M/SL ratio of 2.35 mL/g). A similar behavior was observed for particles obtained with staged shape template polymerization.[8] Wang et al.[8] reported that the RPH of columns packed with monodisperse poly(S-DVB) particles obtained by staged shape template polymerization was mostly between 20 and 25. In our case, RPHs lower than these values were, particularly in the range of 0.25–2.0 mL/min, for the column materials synthesized with the M/SL ratio of 3.0 mL/g and DBP/SL ratios of 1.0 and 1.5 mL/g.

For the columns synthesized with different DBP/SL ratios, the SEC calibration curves are given in Figure 10. The calibration curve of the packing material obtained with the M/SL ratio of 3.0 mL/g and the DBP/SL ratio of 1.0 mL/g was linear in the molecular weight range of 2.1×10^3 and 5×10^6 [Fig. 10(A)]. For particles produced with the M/SL ratio of 3.0 mL/g and DBP/SL ratios of 1.5 and 2.0 mL/g, there were two regions in each calibration curve. The molecular weight of 10^5 was the boundary separating these two regions. However, these curves can also be used for the molecular weight determination in the range of 2.1×10^3 to 5×10^6. In the SEC calibration curves of the particles produced with the M/SL ratio of 2.35 mL/g, two different regions could be more clearly observed [Fig. 10(B)]. In the molecular weight region of 1×10^2 to 1×10^5, the curves were reasonably sharper than the calibration curves of the materials produced with the M/SL ratio of 3.0 mL/g. As shown in the SEM and TEM photographs in Figures 4 and 5, the particles produced with the M/SL ratio of 2.35 mL/g had relatively larger macropores. For this reason, these particles should not be evaluated as suitable packing materials for the molecular weight determination, particularly in the low-molecular-weight range. In conclusion, the particles produced with the M/SL ratio of 3.0 mL/g and all the selected DBP/SL ratios seem more appropriate packing materials for SEC applications in the molecular weight range of 10^3 to 10^6.

The porosity properties of the particles, calculated on the basis of the retention volumes of the standards

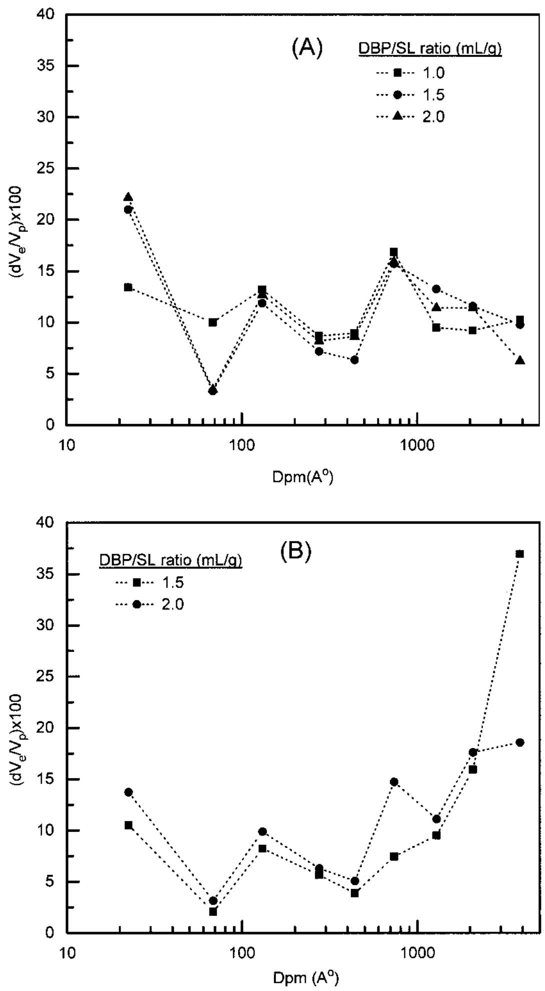

Figure 11 Pore size distribution curves of the particles calculated with the retention volumes of the standards in the SEC study. The M/SL ratios were (A) 3.0 and (B) 2.35 mL/g.

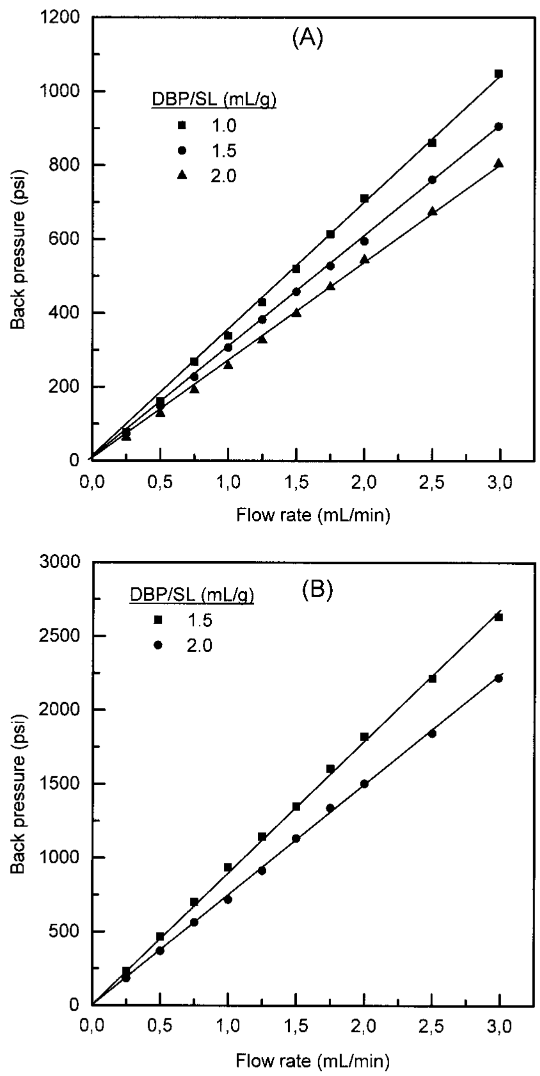

Figure 12 Effect of the flow rate on the back-pressure of columns packed with particles obtained with different DBP/SL ratios (column, 300 mm long × 7.8-mm i.d.; mobile phase = THF). The M/SL ratios were (A) 3.0 and (B) 2.35 mL/g.

used in the SEC study, are given in Table V. The pore size distribution curves calculated according to the method used by Ferreira et al.[31] are given in Figure 11. In this figure, dV_e denotes the variation of V_p for an interval in size of given pores of the particles in the column (mL).[31] V_T and D_{pm} are the total pore volume of the particles in the column (mL) and the representative pore diameter for the pores in certain size intervals (Å), respectively.[31] Therefore, the pore size distribution was given by the ratio dV_e/V_T. As shown in Table V, the mean pore size decreased and the porosity increased as the DBP/SL ratio increased. As shown in Figure 11(A), pores in the size range of 20–4000 Å were found in the particles obtained with the M/SL ratio of 3.0 mL/g. In this range, the 20- and 750-Å pores seemed more dominant than the other sizes. However, the existence of pores larger than 750 Å could be clearly seen in the pore size distribution of particles obtained with the M/SL ratio of 2.35 mL/g [Fig. 11(B)]. On the basis of the results given in Table V and Figure 11, we can conclude that the tendencies observed in the SEM and TEM photographs are also supported by the porosity characteristics determined by SEC.

The effect of the mobile-phase (i.e., THF) flow rate on the back-pressure is given in Figure 12. For all packing materials, the back-pressure varied linearly with the flow rate. At a constant flow rate, the back-pressure decreased with an increasing DBP/SL ratio. This case was valid for the particles produced with both M/SL ratios. This behavior can probably be explained by the higher porosity of the packing material obtained with a higher DBP/SL ratio. However, the particles obtained with the M/SL ratio of 2.35 mL/g exhibited reasonably higher back-pressures than those with the M/SL ratio of 3.0 mL/g. To explain the high back-pressure of the particles obtained with the M/SL ratio of 2.35 mL/g, we examined the particles taken from the columns with an electron microscope. This examination showed the presence of broken particles probably due to the pressure applied during the packing or chromatographic use. An SEM photograph of

A B

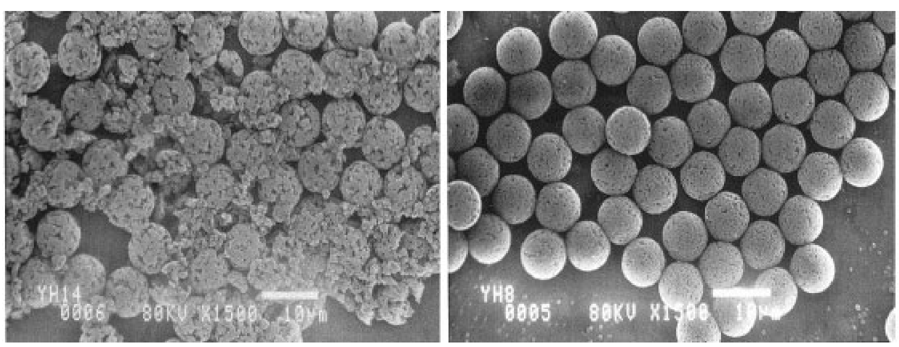

Figure 13 SEM photographs of particles after chromatography: (A) TG14, including broken particles, and (B) TG8, with good mechanical stability (original magnification = 1500×).

the broken particles is given in Figure 13. Here, an SEM photograph of the particles protecting their structural integrity during the chromatographic use (i.e., the particles with good mechanical stability) is also included. The breaking of the particles should result in a reduction in the column porosity, which probably provides higher back-pressure. The presence of craterlike pores in the particle structure may be a reason for the poor mechanical stability of the particles produced with the M/SL ratio of 2.35 mL/g.

CONCLUSIONS

With a relatively new polymerization method called modified seeded polymerization, the effects of the diluent/seed-latex ratio on the size and porosity properties of monodisperse particles were investigated. The monodisperse macroporous particles obtained with different M/SL and DBP/SL ratios were used as packing materials in HPLC. RPC and SEC studies showed that the particles produced with the M/SL ratio of 3.0 mL/g and DBP/SL ratios of 1.0–1.5 mL/g exhibited satisfactory chromatographic performance.

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