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Hydroxyl Functionalized Uniform-Porous Beads, Synthesis and Chromatographic Use

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Uniform-porous poly(dihydroxypropyl methacrylate-co-ethylene dimethacrylate), poly(DHPM-co-EDM) particles were synthesized as an alternative packing material for reversed phase chromatography. In the synthesis, poly(glycidyl methacrylate-ethylene dimethacrylate), poly(GMA-co-EDM) particles were obtained by a multi-stage swelling and polymerization protocol, the so called “modified seeded polymerization”. For this purpose, 2.4 μm polystyrene seed particles were first swollen by dibutyl phthalate (DBP) and then by a monomer mixture including glycidyl methacrylate and ethylene dimethacrylate. The repolymerization of monomer phase in the swollen seed particles provided porous uniform particles approximately 7 μm in size. Poly(DHPM-co-EDM) particles were obtained by the acid hydrolysis of the particles synthesized with different GMA feed concentrations. These particles were used as column-packing material in the reversed phase separation of alkylbenzenes. The retention factor-acetonitrile concentration diagrams clearly showed that the polarity of packing material could be controlled by changing the GMA feed concentration in the “modified seeded polymerization”. The packing materials with more hydrophobic character (i.e., poly(EDM) and poly(DHPM-co-EDM) particles produced with the GMA feed concentrations up to 20%) exhibited better chromatographic performance in the reversed phase mode.

Keywords reversed phase chromatography, HPLC, uniform and porous particles, chromatographic packing, glycidyl methacrylate, ethylene dimethacrylate

Introduction

Polymer based “uniform-porous particles” are an effective packing material family in the HPLC applications. The “activated swelling method” was proposed by Ugelstad et al. for

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the synthesis of uniform particles in the range of 1–20 μm (1–5). The uniform-porous particles 10 μm in size were successfully prepared via seeded emulsion polymerization by Cheng et al. (6, 7). The polar particles obtained by the hydrolysis of uniform poly(glycidyl methacrylate-co-ethylene dimethacrylate) beads from “Staged Shape Template Polymerization” exhibited a good chromatographic performance in the normal phase chromatography (8–10). Lower column back-pressure, more regular flow regime in the column, and the liquid chromatograms with higher resolutions are the main advantages of the chromatographic packings based on uniform particles (11–13). Alternatively, a single stage suspension polymerization method was also proposed for the synthesis of polydisperse poly(dihydroxypropyl methacrylate-co-ethylene dimethacrylate), poly(DHPM-co-EDM) particles suitable for chromatographic use (14). The particles obtained with this protocol were successfully used as packing material in the normal phase mode and provided reasonably high column efficiency (14).

Ogino et al. developed a single stage swelling and polymerization protocol for the synthesis of uniform poly(styrene-divinylbenzene) beads by starting from the polystyrene particles obtained by dispersion polymerization (15). This approach was also used for the synthesis of oligo(ethylene glycol) dimethacrylate beads in the size range of 5.5–9.7 μm (16). These beads were successfully used in the separation of proteins by aqueous-size exclusion chromatography (SEC) (16). Recently, a single step swelling and polymerization protocol was also used for the synthesis of uniform porous glycidyl methacrylate-ethylene dimethacrylate copolymer beads (17, 18). Weak cation exchanger beads and relatively hydrophobic beads synthesized from the copolymer beads were successfully used in protein separation by ion exchange chromatography, and hydrophobic interaction chromatography (17, 18). Zhang et al. proposed a dispersion polymerization method for the synthesis of poly(glycidyl methacrylate-co-divinylbenzene) beads, 3 μm in size (19, 20). The quaternary ammonium and sulfonic acid carrying forms of these beads exhibited a good chromatographic performance both in micro-HPLC and capillary electrochromatography (CEC) (19, 20).

In the last five years, we focused on the synthesis of porous uniform particles suitable as HPLC packing. We tried a multistage polymerization protocol, the so called “modified seeded polymerization” in our laboratory. This method was first used in the synthesis of uniform and macroporous poly(styrene-co-divinylbenzene) particles (21, 22). In the next stage, the uniform porous particles carrying polar functional groups like hydroxyl, carboxyl, epoxypropyl and boronic acid were obtained (23–27). The uniform and porous poly(styrene-co-divinylbenzene) particles produced by “modified seeded polymerization” exhibited a good performance both in the gel permeation and reversed phase chromatography (28, 29). Finally, we extended our method for the synthesis of uniform-porous poly(GMA-co-EDM) beads and aimed to obtain relatively polar stationary phases for HPLC. For this purpose, poly(GMA-co-EDM) beads synthesized with different GMA feed concentrations were converted to poly(DHPM-co-EDM) form by the acid hydrolysis. Although poly(DHPM-co-EDM) beads were mostly used as packing material in the normal phase mode, no attempt has been made for the use of same particles in the reversed phase applications. We thought that poly(DHPM-co-EDM) beads particularly with low DHPM content could be suitable for reversed phase HPLC (RPLC). For this reason, we synthesized poly(DHPM-co-EDM) particles with different DHPM contents (i.e., with different polarities) and tried them as chromatographic support in RPLC. Here, we wish to report the synthesis and chromatographic performance of the poly(DHPM-co-EDM) particles obtained by our modified seeded polymerization protocol.

Experimental

Materials

Glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDM) were supplied from Aldrich Chem. Corp. and used without further purification. The properties and suppliers of other chemical used in this study were given elsewhere (28, 29).

Particle Synthesis

The polystyrene seed latex 2.4 μm in size was synthesized by dispersion polymerization based on the principles followed in our previous studies (28–31). In a typical synthesis of uniform poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(GMA-co-EDM) particles, by “modified seeded polymerization”, dibutylphthalate, (DBP, 8.0 mL) was emulsified in the aqueous medium (120 mL) including 0.25% (w/w) sodium lauryl sulfate (SLS) as the emulsifier by sonication for 30 min. The latex dispersion (approximately 3.5 mL) including polystyrene (PS) seed particles (0.6 g) was added to the DBP emulsion. The resulting dispersion was stirred magnetically (400 rpm) at room temperature for 24 h for the absorption of DBP by PS seed particles. In the next stage, a monomer phase comprised of GMA (2.6 mL), EDM (10.4 mL) and benzoyl peroxide (BPO, 0.50 g) was emulsified in water (120 mL) including 0.25% (w/w) SLS. Monomer emulsion was then mixed with the aqueous emulsion containing DBP-swollen seed particles. The resulting emulsion was stirred at room temperature for 24 h at 400 rpm for the absorption of monomer phase by the DBP-swollen seed particles. Following this step, an aqueous solution (28 mL) including 5.0% (w/w) polyvinyl alcohol (PVA) was added into the resulting dispersion and the medium was purged with nitrogen for 5 min. Repolymerization in the swollen seed particles was conducted at 70°C and 120 cpm shaking rate for 24 h. The monodisperse-porous particles approximately 7.0 μm in size were obtained as the product. In the particle synthesis experiments, the GMA feed concentration in the monomer phase was changed between 0–60% (v/v).

The isolated particles were washed with ethanol several times and extracted with tetrahydrofuran (THF) according to the procedure given elsewhere (28, 29). The hydrolysis of poly(GMA-co-EDM) particles was performed in 0.5 M H_2SO_4 solution at 60°C for 3 h (13). The hydrolyzed particles were extensively washed with distilled water and stored at room temperature.

The size distribution properties of the particles and the surface morphology were investigated by scanning electron microscopy (SEM, JEOL, JEM 1200EX, Japan) by following a procedure described in detail elsewhere (28, 29). The porosity properties of the particles were determined by Size Exclusion Chromatography (SEC) according to the literature (14).

Chromatographic Study

The particles were slurry packed into 250 mm \times 4.6 mm I.D. HPLC columns by following the protocol given elsewhere (28, 29). Size-Exclusion Chromatography (SEC) was performed by using stainless steel columns 250 \times 4.6 mm i.d. with THF as the mobile-phase. The chromatograms were recorded by a SPD-10 AVVP UV (Shimadzu, Japan) detector at 254 nm. The back pressures of the column were determined by varying the mobile-phase (i.e., THF) flow-rate in the range of 0.25–2.0 mL/min.

The separation of alkylbenzenes (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene and pentylbenzene) was studied in isocratic mode by using acetonitrile-water mixture as the mobile-phase. In these experiments, the acetonitrile concentration and the flow rate of mobile phase were changed. The liquid chromatograms were recorded by a UV-detector operated at 254 nm. For a certain alkyl benzene, the retention factor (k) was calculated based on Equation (1), where V_a and V_v are the retention time of the selected alkylbenzene in acetonitrile-water mixture and retention time of the void volume marker. The peak resolution and the theoretical plate number were calculated based on the conventional expressions (28, 29).

$$k = (V_a - V_v)/V_v \quad (1)$$

Results and Discussion

Particle Characterization

We used the term “modified seeded polymerization” because a different multi-stage polymerization procedure with respect to the conventional original methods, “activated swelling protocol” and “Ogino’s method” was followed in our case (6, 7, 15, 16, 28, 29). Our multi-stage particle-synthesis protocol was started with a seed latex obtained by dispersion polymerization. The sufficiently low MW of the seed latex allowed the use of seed material as a polymeric porogen with relatively high concentration in the multi-stage synthesis protocol. For this reason, the monomer/seed latex ratio was set to 22.5 mL/g. The concentration of seed latex in the monomer mixture was reasonably high (i.e., 4.4% w/w). On the other hand, the swelling activator (i.e., DBP) was also used as porogenic solvent. Although DBP had a linear structure, it worked well when only EDM or a mixture of GMA-EDM with relatively low GMA content was used as the monomer phase.

The properties of seed latex are given in Table 1. The coefficient of variation, (CV, 2.2%) indicated that the seed particles were nearly uniform. In the synthesis of poly(glycidyl methacrylate-co-ethylene dimethacrylate), poly(GMA-co-EDM) particles, GMA feed concentration was changed between 0 and 60% v/v by keeping the other conditions as constant (Table 2). Poly(dihydroxypropyl methacrylate-co-ethylene dimethacrylate), poly(DHPM-co-EDM) particles were obtained by the acid hydrolysis of the polymerization product (i.e., poly(GMA-co-EDM) particles). Hence, the uniform-porous particles with different polarities were synthesized by converting the epoxypropyl groups into the diol form. A representative SEM photograph exemplifying the average size and size distribution of poly(DHPM-co-EDM) particles is given in Figure 1. As also seen

Table 1
The properties of polystyrene seed latex

Seed latex code	SLC
Polymerization yield (% w/w)	81
Average particle size (D_n , μm)	2.4
Coefficient of variation (%)	2.2
Weight average molecular weight (M_w)	5.7×10^4

Table 2
The synthesis conditions of monodisperse-porous particles

Particle type	Seed (g)	DBP (mL)	GMA (mL)	EDMA (mL)	BPO (mL)	PVA (mL)
DHPM0	0.6	8.0	0	13	0.5	1.4
DHPM10	0.6	8.0	1.3	11.7	0.5	1.4
DHPM20	0.6	8.0	2.6	10.4	0.5	1.4
DHPM40	0.6	8.0	5.2	7.8	0.5	1.4
DHPM60	0.6	8.0	7.8	5.2	0.5	1.4

here, the acidic hydrolysis caused no deformation in the spherical form. The size distribution properties calculated based on the SEM photographs are given in Table 3. As seen here, the average size slightly decreased with increasing GMA feed concentration. The size distribution was relatively narrower for the particles synthesized with the GMA feed concentrations higher than 20% v/v.

The SEM photographs showing the detailed surface morphology of poly(DHPM-co-EDM) particles are given in Figure 2. As seen here, the particles with larger pores were obtained with higher GMA feed concentration. The porosity properties of poly(DHPM-co-EDM) particles determined based on a SEC study according to the method proposed by Ferreira et al. are given in Table 3 (23). As seen here, higher average pore size was also measured for the particles produced with higher GMA feed concentration (Table 3). On the other hand, the porosity and particularly the specific surface area of poly(DHPM-co-EDM) particles significantly decreased with increasing GMA feed concentration (Table 3).

The effect of GMA feed concentration on the porous structure of particles may be explained by considering the pore-formation mechanism proposed for the synthesis of

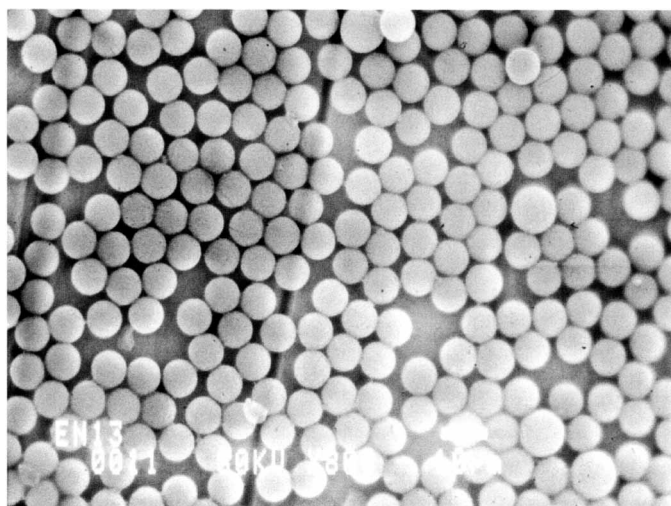


Figure 1. A representative SEM photograph showing the size and size distribution of poly(DHPM-co-EDM) particles obtained with the GMA feed concentration of 20% v/v, Magnification, 800X.

Table 3
The size distribution and porosity properties of particles

GMA concentration (% v/v)	Particle size (μm)	CV (%)	Average pore size (nm)	Pore volume (mL/g)	Porosity (% v/v)	Surface area (m^2/g)
0	7.2	8.2	46	0.70	47	61.5
10	7.1	9.3	49	0.67	45	55.3
20	7.4	5.0	56	0.68	46	49.0
40	7.0	4.0	82	0.64	42	31.6
60	7.0	4.4	97	0.64	42	26.5

uniform particles with multistage swelling and polymerization protocols (9, 10). According to this mechanism, the first stage in the pore formation process was the generation of crosslinked gel microspheres in the forming particles (9, 10). The second stage was the agglomeration and fixation of these gel microspheres. In the case of higher GMA concentration, the formation of larger pores may be explained as follows, the first possibility is the formation of larger crosslinked gel microspheres during the first stage of the pore formation process. Hence, larger fixed blocks are generated by the agglomeration of larger gel spheres. The second possibility is the extensive agglomeration of the crosslinked gel microspheres in the presence of a relatively polar monomer (i.e., GMA) in the second stage of the pore-formation. This case also involves the formation of larger fixed blocks within the forming particles. Both cases should involve the formation of larger voids filled with the porogen (i.e., pores) between the fixed blocks

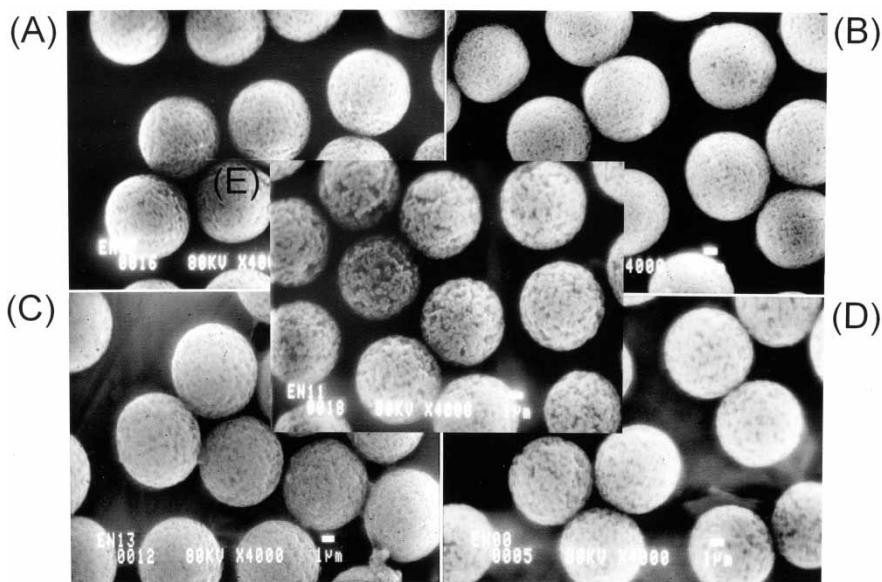


Figure 2. The SEM photographs showing the surface morphology of poly(DHPM-co-EDM) particles obtained with different GMA feed concentrations. GMA feed concentration (% v/v), (A) 0, (B) 10, (C) 20, (D) 40, (E) 60. (Magnification, 4000X).

in the beads produced with relatively higher GMA feed concentration. A similar behaviour was also observed in our previous study performed with different polar acrylic monomers like 2-hydroxyethylmethacrylate acrylic acid and GMA (24). It should be also noted that the decrease in the porosity may also lead to a decrease in the average particle size. Hence the decrease in the average size may be explained by the porosity decrease with increasing GMA concentration.

After packing of poly(DHPM-co-EDM) particles synthesized by starting from different GMA feed concentrations into the HPLC columns, the variation of column back-pressure with the mobile phase flow rate was determined using THF as the mobile phase (Figure 3). As seen here, the particles obtained with higher GMA feed concentration provided higher column back-pressure at constant flow rate. Based on the Ergun equation, the pressure drop of a packed bed column increases with the decreasing porosity of the packing material (32). Hence, the lower porosity of the particles synthesized with higher GMA feed concentration is a factor contributing to higher back pressure. The lower size of particles obtained with higher GMA concentration should be another factor contributing to an increase in the back pressure.

Chromatographic Evaluation

In this part, the liquid chromatograms were obtained by the isocratic separation of alkyl-benzenes by using acetonitrile (AcN)-water mixture as the mobile phase. For each column, isocratic separations were performed by changing the AcN feed concentration in a suitable range. The chromatograms obtained with the AcN concentration of 40% v/v are exemplified for all columns in Figure 4. Poly(DHPM-co-EDM) particles with more polar character

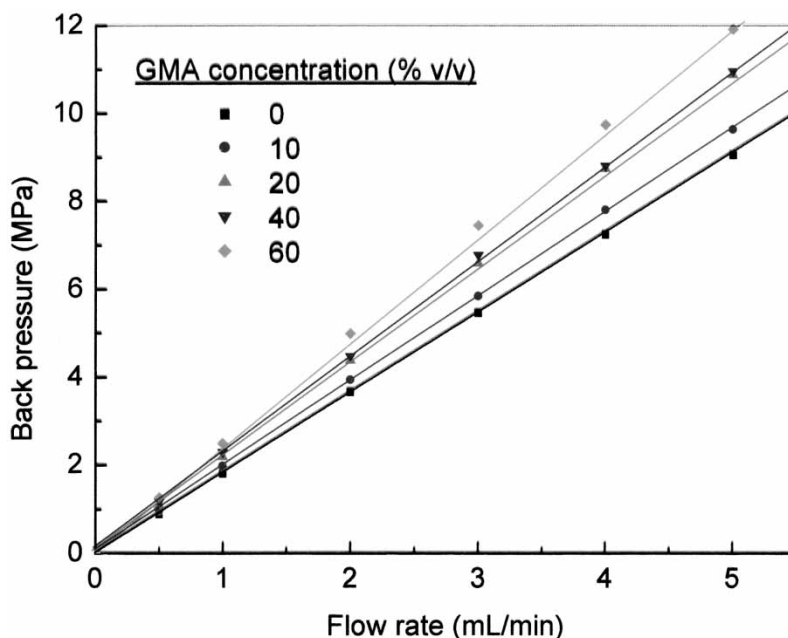


Figure 3. The variation of back-pressure with the mobile phase flow rate for the columns packed with poly(DHPM-co-EDM) particles obtained with different GMA feed concentrations. Column, 250 × 4.6 mm i.d., mobile phase, THF.

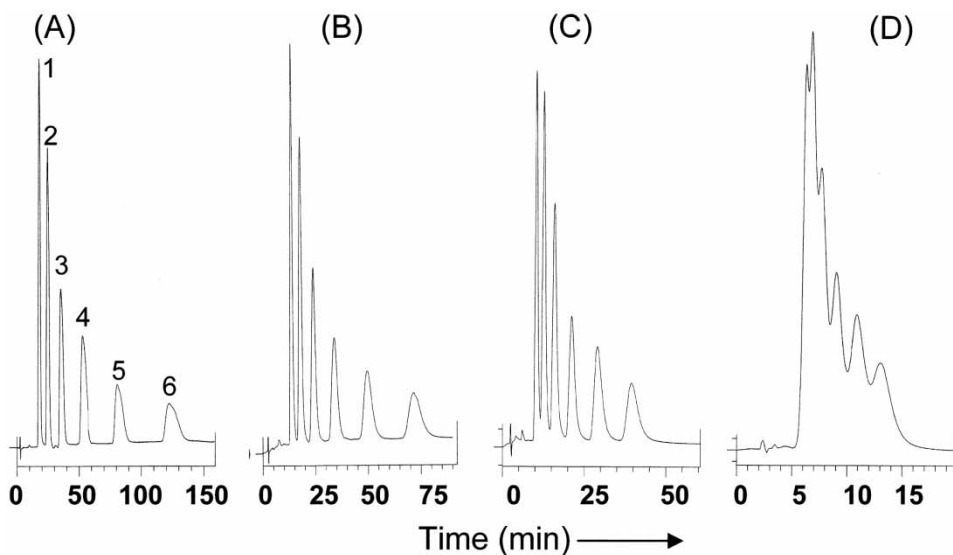


Figure 4. The liquid chromatograms obtained for the isocratic separation of alkylbenzenes in the columns packed with poly(DHPM-co-EDM) particles synthesized with different GMA feed concentrations. GMA feed concentration (% v/v), (A) 0, (B) 10, (C) 20, (D) 40, Mobile phase, AcN-Water, AcN concentration in the mobile phase, 40% v/v, Column size, 250 × 4.6 mm i.d, UV detection at 254 nm. Flow rate, 1 mL/min, Order of peaks, (1) Benzene, (2) Toluene, (3) Ethylbenzene, (4) Propylbenzene, (5) Butylbenzene, (6) Pentylbenzene.

should be obtained by starting from higher GMA concentration. As seen in Figure 4, the chromatographic separation was performed in a shorter time in the columns with higher polarity. It should be noted that the isocratic separation of alkylbenzenes by using AcN-water as the mobile phase could not be achieved with the column packed with poly(DHPM-co-EDM) particles produced with the GMA feed concentration of 60% v/v.

The resolution values calculated based on the chromatograms taken with the AcN concentrations ranging between 20–60% v/v are presented in Table 4, where $R(n + 1/n)$ is the resolution between peak $n + 1$ and peak n . The resolution of 1.5 is commonly accepted as the threshold value showing the satisfactory chromatographic separation of two successive analytes. In the evaluation of chromatographic behaviours of the columns, the resolution should be considered together with the total analysis time. For this reason, the total analysis time (TAT) values were included in Table 4. Here, TAT was defined as the time necessary for completion of the chromatographic separation of the alkylbenzene mixture including six homologs. With the AcN concentration of 50% v/v, the column including poly(EDM) particles provided satisfactorily high resolutions in a reasonable analysis period (i.e., 50 min). For the poly(DHPM-co-EDM) particles prepared with the GMA feed concentrations of 10 and 20%, a similar behavior was observed with the AcN concentration of 40%. With low AcN concentrations (<30% v/v), poly(DHPM-co-EDM) particles synthesized with the GMA feed concentrations of 20 and 40% v/v could provide high resolutions with extremely long analysis times (i.e., >200 min). For these columns having higher polarity with respect to the others, the increase in the AcN concentration (i.e., a decrease in the polarity of mobile phase) led to more drastic decreases in the chromatographic resolution.

Table 4

The resolutions in the chromatograms obtained with different AcN feed concentrations

AcN% (v/v)	TAT (min)	Resolutions				
		R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
Poly(EDM)						
40	160	1.66	2.02	2.46	2.79	2.71
50	50	1.53	1.64	1.83	1.99	1.95
60	20	0.91	1.03	1.25	1.35	1.26
GMA, 10%						
40	100	1.83	1.91	2.16	2.40	2.12
50	30	1.05	1.21	1.39	1.52	1.45
60	15	<0.50	<0.50	<0.50	<0.50	<0.50
GMA, 20%						
30	200	2.05	2.40	2.99	3.10	3.05
40	60	1.31	1.50	1.80	1.89	1.78
50	15	0.60	0.72	0.94	0.86	0.84
GMA, 40%						
20	375	1.61	1.65	2.11	2.36	2.23
30	80	1.06	1.11	1.36	1.56	1.52
40	20	<0.50	<0.50	<0.50	<0.50	<0.50

For the columns packed with the particles having different polarities, the variation of retention factor with the AcN concentration is given in Figure 5. In our study, although relatively polar stationary phases with respect to the common packings were used in the reversed phase separation, a linearity between the retention factor and AcN concentration was also observed. At constant AcN concentration, the lower retention factor values for the columns produced with higher GMA feed concentrations was clear evidence for the higher column polarity.

In Figure 6, the retention factor was plotted against the number of alkyl carbon atoms for the columns with different polarities. The data collected with the AcN concentration of 40% was used in this figure. The slope of the retention factor-AcN concentration plot shown in the graph is a good indicator showing the hydrophilicity (i.e., polarity) of the packing material. It should be noted that a better chromatographic separation is possible in a column with higher slope since the difference in the retention times of two successive homologs is greater in the presence of higher slope. The slopes of the straight lines belonging to the poly(DHPM-co-EDM) beads were shown on the plot. As seen here, the slope markedly increased with the decreasing DHPM content of the porous particles. The lowest slope (i.e., 0.093) was obtained with the most polar packing material at the AcN concentration of 40% (i.e., the poly(DHPM-co-EDM) beads prepared with the GMA feed concentration of 40% v/v). As seen in Figure 4, a successful chromatographic separation could not be achieved with the AcN concentration of 40% in the column containing poly(DHPM-co-EDM) particles prepared with the GMA feed concentration of 40%. Hence, the packing-material with such a lower slope should

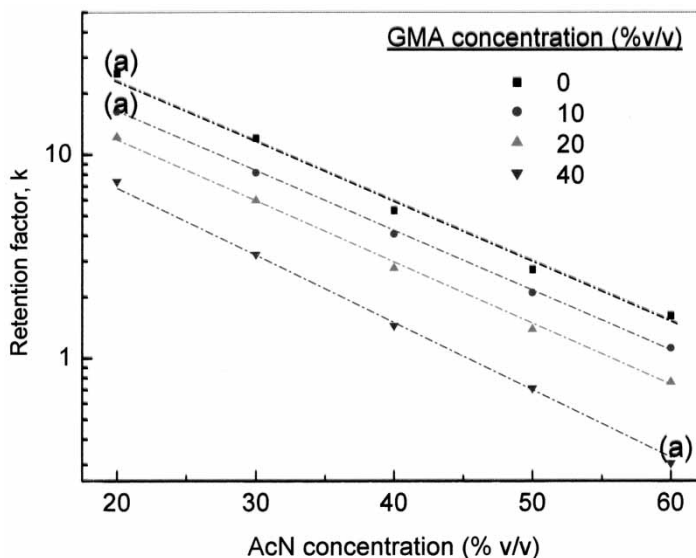


Figure 5. The variation of retention factor with the AcN concentration for the columns packed with poly(DHPM-co-EDM) particles synthesized with different GMA feed concentrations. Analyte, Benzene, (a), These points were found by single analyte injection, For chromatographic conditions see Figure 4.

not be considered as suitable for the isocratic separation of alkylbenzenes. This graph again indicated that the column polarity was controlled by changing the GMA feed concentration in the “modified seeded polymerization”.

The results indicated that our polymerization protocol was particularly suitable for the synthesis of uniform-porous poly(EDMA) and poly(GMA-co-EDM) particles with

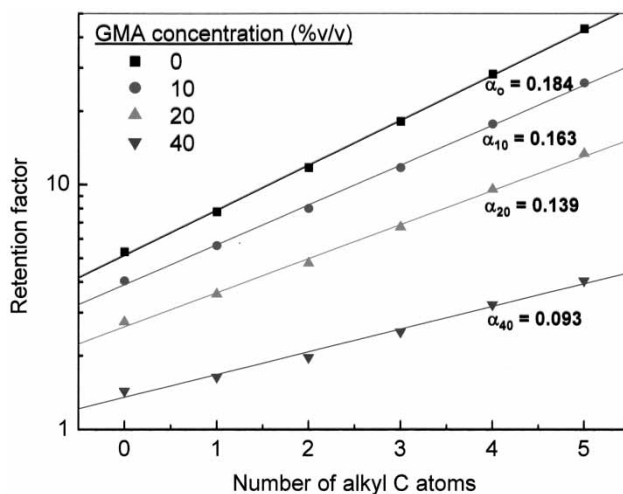


Figure 6. The variation of retention factor with the number of alkyl carbon atoms for the columns packed with poly(DHPM-co-EDM) particles synthesized with different GMA feed concentrations. AcN concentration in the mobile phase, 40% v/v, For other chromatographic conditions see Figure 4.

relatively lower GMA content (i.e., up to 20% v/v). Indeed, this idea was supported by the effect of GMA feed concentration on the porosity properties of our particles. As seen in Table 3, the average pore size is significantly increased and the specific surface area markedly decreased for the poly(DHPM-co-EDM) particles synthesized with relatively high GMA feed concentrations (i.e., 40 and 60% v/v). In the case of higher GMA feed concentration, the aggregation tendency of crosslinked gel microspheres is probably higher due to relatively polar character of the selected porogen (i.e., DBP) which in turn results in the formation of particles with relatively larger pores and low specific surface area.

The effect of AcN concentration on the theoretical plate number (TPN) of the columns with different polarities is shown in Figure 7. As seen here, relatively higher TPN values were obtained with the poly(DHPM-co-EDM) particles prepared with the GMA feed concentrations of 10 and 20%. In other words, the column efficiency increased with the increasing polarity of the stationary medium up to 20% of GMA feed concentration. The low TPN values observed for the poly(DHPM-co-EDM) particles synthesized with a GMA feed concentration of 40% is probably explained by insufficient porosity properties of this particle type.

In order to test the effect of flow rate on the chromatographic behavior of the packing materials, the AcN concentration providing satisfactory resolutions with a reasonable analysis time was used for each column. Based on this principle, the column including poly(EDM) particles was operated with the AcN concentration of 50%. The AcN concentration of 40% was used for the poly(DHPM-co-EDM) based columns prepared with the GMA feed concentrations of 10 and 20% v/v. For the particles synthesized with the GMA feed concentration of 20%, the liquid chromatograms obtained with different flow rates ranging between 0.5 and 3.0 mL/min are exemplified in Figure 8. For all packing materials, the resolutions calculated based on the chromatograms obtained with different flow rates are presented in Table 5. Although with a six-fold increase in the flow rate, the resolution power was approximately preserved for all columns tried in

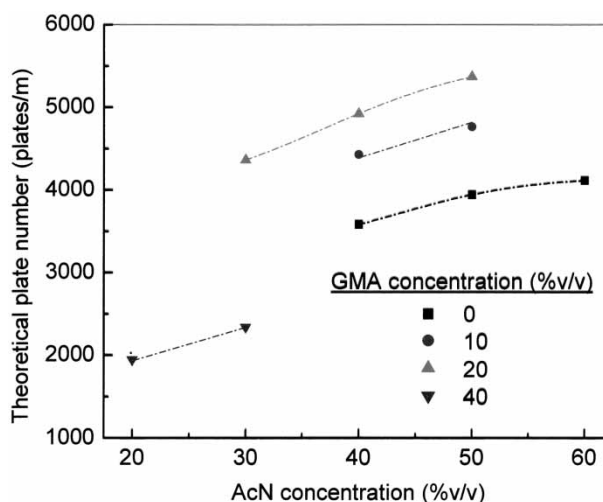


Figure 7. The variation of theoretical plate number with the AcN concentration. Analyte, Benzene, For other chromatographic conditions see Figure 4.

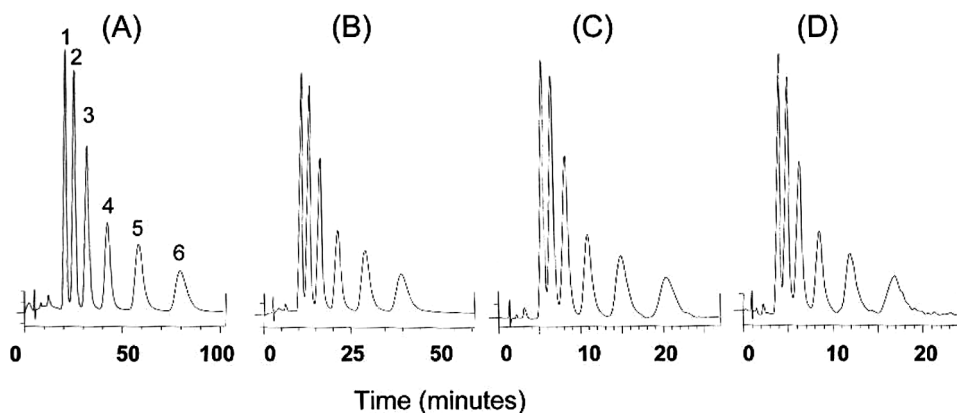


Figure 8. The liquid chromatograms obtained with different flow rates for the isocratic separation of alkylbenzenes in the columns packed with poly(DHPM-co-EDM) particles synthesized with the GMA feed concentration of 20% v/v. Flow rate (mL/min), (A) 0.5, (B) 1.0, (C) 2.0, (D) 3.0, Mobile phase, AcN-Water, Column size, 250 × 4.6 mm i.d., UV detection at 254 nm. Order of peaks, (1) Benzene, (2) Toluene, (3) Ethylbenzene, (4) Propylbenzene, (5) Butylbenzene, (6) Pentylbenzene.

Table 5
The resolutions calculated based on the chromatograms obtained with different flow rates

Flow rate, mL/min	Resolutions				
	R(2/1)	R(3/2)	R(4/3)	R(5/4)	R(6/5)
Poly(EDM)					
0.50	1.55	1.80	2.01	2.19	2.14
0.75	1.50	1.67	1.92	2.13	2.03
1.00	1.53	1.64	1.83	1.99	1.95
2.00	1.30	1.53	1.65	1.78	1.78
3.00	1.42	1.58	1.57	1.74	1.85
GMA, 10%					
0.50	1.66	2.02	2.34	2.61	2.17
0.75	1.70	1.91	2.17	2.34	2.27
1.00	1.83	1.91	2.16	2.40	2.12
2.00	1.63	1.67	2.03	1.93	2.11
3.00	1.47	1.70	2.08	1.97	1.74
GMA, 20%					
0.50	1.57	1.72	2.00	2.19	2.08
0.75	1.68	1.92	2.22	2.23	1.99
1.00	1.31	1.50	1.80	1.89	1.78
2.00	1.34	1.44	1.67	1.67	1.62
3.00	1.32	1.41	1.70	1.73	1.59

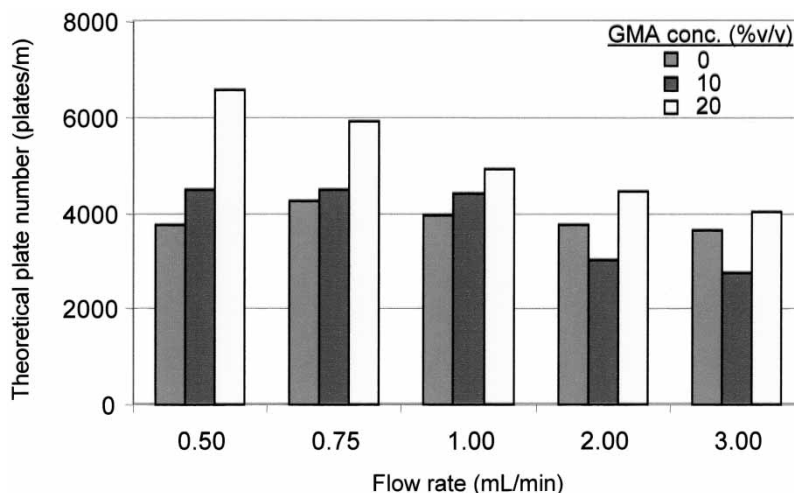


Figure 9. The variation of theoretical plate number with the AcN concentration. Analyte, Benzene, For other chromatographic conditions see Figure 8.

this set. At constant flow rate, the poly(DHPM-co-EDM) particles prepared with the GMA feed concentration of 10% provided the highest resolutions in most cases.

The variation of theoretical plate number with the flow rate is given in Figure 9 for the poly(EDM) and poly(DHPM-co-EDM) particles synthesized with the GMA feed concentrations of 10 and 20%. These values were determined by using benzene as the analyte on the chromatograms taken with the flow rates ranging between 0.5 and 3.0 mL/min. For the column packed with poly(EDM) particles, no significant change was observed in TPN with increasing flow rate. However TPN is slightly decreased with flow rate for the poly(DHPM-co-EDM) columns prepared with the GMA feed concentrations of 10 and 20%. Slight decrease in the column efficiency is probably explained by the effect of pressure driven intraparticle convection taking place in the beads with an average pore size larger than 40 nm. At constant flow rate, higher TPN values were observed with the increasing column polarity. Because shaper peaks were obtained with more polar columns since the affinity of apolar analytes (i.e., alkylbenzenes) to the packing material was relatively lower. The particles prepared with the GMA feed concentration of 20% provided the highest TPN values. With this packing material, the TPNs close to 7000 plates/m could be achieved by using benzene as the analyte with relatively low flow rates (i.e., 0.5–1.0 mL/min).

Conclusions

The uniform-porous poly(EDM) and poly(DHPM-co-EDM) particles were synthesized by “modified seeded polymerization”. The effects of GMA feed concentration on the porosity properties and the reversed phase chromatographic behaviors of particles were investigated. The results showed that poly(EDM) particles and poly(DHPM-co-EDM) particles synthesized with the GMA feed concentrations up to 20% exhibited satisfactory chromatographic performance in the isocratic separation of alkylbenzenes by reversed phase chromatography.

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