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Aqueous size exclusion chromatography in semimicro and micro-columns by newly synthesized monodisperse macroporous hydrophilic beads as a stationary phase

Çiğdem Gölgelioğlu[a], Aslıhan Bayraktar[a], Bekir Çelebi[a], Erdal Uğuzdoğan[b], Ali Tuncel[a,*]

[a] Hacettepe University, Chemical Engineering Department, Ankara, Turkey
[b] Pamukkale University, Chemical Engineering Department, Ankara, Turkey

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ABSTRACT

A new class of monodisperse macroporous beads in the hydrophilic form were synthesized by seeded microsuspension copolymerization of two acrylic crosslinking agents, glycerol dimethacrylate (GDMA) and glycerol-1,3-diglycerolate diacrylate (GDGDA). The monodisperse porous poly(glycerol dimethacrylate-*co*-glycerol-1,3-diglycerolate diacrylate), poly(GDMA-*co*-GDGDA) beads were highly hydrophilic in nature due to hydroxyl functionality resulting from both crosslinking agents. The beads with different particle sizes between 4.5 and 6.7 μm and with different porous properties were obtained by changing the seed latex to monomer ratio. The bead size decreased, the average pore size increased and the specific surface area decreased with increasing seed latex to monomer ratio. Poly(GDMA-*co*-GDGDA) beads were slurry packed in microbore and semimicro-HPLC columns and successfully used as a stationary phase in aqueous size exclusion chromatography (SEC) mode in a micro-liquid chromatography system. The aqueous SEC runs were performed by using dextran standards in the molecular weight range of 1000–670,000 Da. SEC calibration curves exhibiting linearity in a wider range of molecular weight were obtained with the semi-micro and micro-HPLC columns packed with the poly(GDMA-*co*-GDGDA) beads synthesized with the seed latex to monomer ratios of 0.038 and 0.058 g/mL. The dextran standards could be eluted in an analysis time shorter than 2 min using micro or semi-micro columns packed with poly(GDMA-*co*-GDGDA) beads as stationary medium. These packings are suitable for molecular weight determination between 5×10^3 and 5×10^5 Da in the aqueous medium by using mobile phase flow rates in the range of 25–250 μL/min. The average molecular weight determinations of different water soluble polymers, an ionic polymer, poly(2-dimethylaminoethyl methacrylate), a zwitterionic polymer, poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide), and a non-ionic polymer, poly(vinyl alcohol) were performed on a semimicro-column packed with the poly(GDMA-*co*-GDGDA) beads. Satisfactory results were obtained in the molecular weight determination of hydrophilic polymers by aqueous SEC.

1. Introduction

Important characteristics of stationary media for different chromatographic applications include particle size, pore volume, sorptive properties and matrix rigidity [1]. The research effort on the synthesis of monodisperse macroporous polymer particles as the stationary media for different chromatographic modes is mostly focused on the relatively apolar (i.e. hydrophobic) forms [2]. Staged-shape template polymerization of some acrylate based monomers and crosslinking agents (i.e. glycidyl methacrylate, 2-hydroxethyl methacrylate and ethylene dimethacrylate) is one of the few possible methods that has been successfully used for the synthesis of polar stationary media in the form of monodisperse-porous particles [3–6]. Porous monodisperse poly(methacrylic acid-*co*-ethylene dimethacrylate), poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) and poly(2,3-dihydroxypropyl methacrylate-*co*-ethylene dimethacrylate) beads, which can be used as a platform for the production of separation media, polymeric reagents, and supports, were prepared by using this method [3–6]. In addition, crosslinked, hydrophilic polymer beads or silica based materials can be used as the stationary medium for the SEC analysis of water-soluble molecules such as for example biological molecules [7–9].

Some of the polymerization methods proposed for the synthesis of monodisperse porous beads with hydrophilic surface characteristics mostly included an additional stage for the formation

* Corresponding author.
E-mail address: atuncel@hacettepe.edu.tr (A. Tuncel).

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of a hydrophilic shell layer on the surface after the synthesis of core beads [10–12]. As a typical example, a hydrophilic monomer mixture (glycerol monomethacrylate (GMMA) and glycerol dimethacrylate (GDMA)) was polymerized on the surface of porous poly(styrene-*co*-divinylbenzene) beads for the preparation of a composite bead structure with a hydrophobic core and a hydrophilic shell layer [10]. The modified medium with a hydrophilic layer could be used to analyse a peptide-mixture containing large molecules [10]. One application of these beads was the preparation of very selective chiral separation media for HPLC of enantiomers. A uniform-sized molecularly imprinted polymer for (*S*)-naproxen selectively modified with a hydrophilic external layer has been prepared by a multi-step swelling procedure and thermal copolymerization of 4-vinylpyridine and ethylene dimethacrylate [11]. The hydrophilic external layer was also formed by the copolymerization of GMMA and GDMA during the molecular imprinting [11]. Recently, an aqueous surface-initiated atom transfer radical polymerization (SI-ATRP) was used to grow polymer brushes from a "gigaporous-polar" polymeric chromatography support for use as a novel size exclusion chromatography medium for aqueous media [12]. The hydrophilic crosslinking agent, GDMA was copolymerized with long-chain alkyl methacrylate monomers by a multistep swelling and polymerization method for the synthesis of monodisperse, polymer-based packing materials affording excellent chromatographic performance in semimicro HPLC [13]. The packing materials exhibited a considerable improvement in column efficiency, as demonstrated on the separation of polyaromatic hydrocarbons [13]. However, the obtained material was not hydrophilic in nature due to the long chain alkyl methacrylate units in the structure and hence would not be suitable for the SEC separation of water-soluble polymers.

In the present study, the synthesis of monodisperse macroporous beads by the copolymerization of glycerol dimethacrylate (GDMA) and glycerol-1,3-diglycerolate diacrylate (GDGDA) was optimized for obtaining hydrophilic beads suitable as stationary medium for liquid chromatography. The poly(GDMA-*co*-GDGDA) beads were successfully used as stationary medium in aqueous SEC performed in semi-micro and micro-HPLC system. With respect to the conventional SEC, capillary or microcolumn SEC has various advantages, such as low eluent consumption, ease of interfacing with a mass spectrometer, and ability to prepare long columns [14]. In the case of capillary columns or microcolumns, more efficient separation is possible under optimized conditions with respect to the conventional columns [15,16]. Based on these reasons, we preferred to evaluate the material developed in micro-HPLC.

2. Experimental

2.1. Materials

The monomers, glycidyl methacrylate (GMA, 97% w/w, contains 100 ppm monomethyl ether hydroquinone as inhibitor), glycerol dimethacrylate (GDMA, technical grade, 85%, contains 200 ppm monomethyl ether hydroquinone as inhibitor) and glycerol-1,3-diglycerolate diacrylate (GDGDA, technical grade, contains 1000 ppm monomethyl ether hydroquinone as inhibitor) were supplied from Aldrich Chem. Co., U.S.A. and used without further purification. Cyclohexanol (Cyc-OH), dibutylphthalate (DBP), ethanol (Et-OH, HPLC grade), tetrahydrofuran (THF, HPLC grade) and isoamylalcohol (IAm-OH) were supplied from Aldrich Chem. Co. Sodium lauryl sulfate (SLS), polyvinylalcohol (PVA, 87–89% hydrolysed, molecular weight: 85,000–146,000 Da) and polyvinyl pyrrolidone K-30 (PVP K-30, average molecular weight: 40,000 Da) were obtained from Sigma Chemical Co., U.S.A. 2,2′-Azobisizobutyronitrile (AIBN) was obtained from Merck A.G., Darmstad, Germany. 2-Dimethylaminoethyl methacrylate (DMAEM, contains 2000 ppm monomethyl ether hydroquinone as inhibitor, Aldrich Chem. Co.) was purified by passing through a column filled with alumina to remove inhibitor. 2-(Methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide) (MEDSPAH), anisole, CuCl (99%), CuBr (98%), 1,1,4,7,10,10-hexamethyltriethylene tetramine (HMTETA), bipyridyl (Bpy) and ethyl 2-bromoisobutyrate (EBriB) were purchased from Aldrich and used as received without further purification. Dextran standard set (Mw: 1000–670,000) and fructose were supplied from Fluka Chemie A.G., Switzerland. Polystyrene standards (Mw: 1000–2,000,000) were obtained from Aldrich Chem. Co.

2.2. Preparation of seed latex

The poly(glycidyl methacrylate), poly(GMA) seed latex 1.4 μm in size was obtained by dispersion polymerization. For this purpose, GMA (3.0 mL) and AIBN (0.06 g) were dissolved in absolute Et-OH (30 mL) containing PVP K-30 (0.50 g) as the stabilizer in a sealed pyrex-glass reactor. The polymerization medium was purged with nitrogen for 3 min and the reactor was heated to 70 °C in a temperature controlled shaking water-bath. Warm up time was 40 min. The polymerization was conducted at 70 °C for 24 h with a shaking rate of 120 cycles per minute. The resulting poly(GMA) latex was cleaned by a successive centrifugation-decanting procedure by using DDI water. The seed particles were then suspended in DDI water.

2.3. Synthesis and characterization of monodisperse poly(GDMA-co-GDGDA) particles

Poly(GDMA-*co*-GDGDA) particles were synthesized by a newly developed multistage swelling and polymerization protocol [17]. In the first stage of the two step swelling and polymerization protocol, the organic phase containing Cyc-OH (3.1 mL) and DBP (1.8 mL) was emulsified in water (60 mL) containing SLS (0.15 g) and PVA (0.8 g) by sonication for 40 min. The suspension containing PGMA seed particles (0.30 g) was thereafter added to the emulsion and magnetically stirred at room temperature for 24 h. In the second stage, the monomer phase containing GDMA (2.6 mL), GDGDA (2.6 mL), BPO (0.12 g) and IAm-OH (0.25 mL) was emulsified in water (60 mL) containing SLS (0.15 g). The emulsion was then mixed with the aqueous suspension of seed particles and thereafter a PVA solution (0.8 g in 10 ml water) was added. The polymerization was conducted at 80 °C at 120 cpm shaking rate for 24 h. The monodisperse porous poly(GDMA-*co*-GDGDA) particles were obtained as the product at the end of the polymerization stage. Particles were washed and extracted in a series of steps using ethanol, THF, ethanol and water. Finally, the particles were re-suspended in DDI water. The size distribution properties and the surface morphology of poly(GDMA-*co*-GDGDA) particles were examined by scanning electron microscopy (Zeiss, Evo-50, Germany).

2.4. Determination of porous properties

The particles were slurry packed into 100 mm × 1.0 mm i.d. or 150 mm × 2.0 mm i.d. stainless steel columns under high pressure (250 bar) by following the protocol given elsewhere [18,19].

The porosity characteristics (i.e. average pore size, pore-size distribution, porosity and pore volume of the particles) were determined by inverse-size exclusion chromatography according to the method used by Ferreira et al. [20]. For this purpose, the retention volumes of toluene and polystyrene (PS) standards with average molecular weights (MW) ranging between 1000 and 2,000,000 Da were determined using THF as the mobile phase in a

conventional HPLC system. PS standards with sufficiently high MWs (ca. 2,000,000 Da) are easily available for the estimation of void volume between particles in the column. In the literature, the relationships between pore size and molecular weight of the standard used are also well established for the PS standards. The pore size value (D_p, nm) corresponding to a certain PS standard was calculated based on Eq. (1) [18]. Where, M_w is the weight average molecular weight of the polystyrene standard.

$$D_p = 0.062M_w^{0.59} \quad (1)$$

In the pore size distribution curve, the variation of pore volume for an interval in size of given pores of the particles in the column was defined by *dVe* (mL) [18,19]. For the generation of pore-size distribution curve, *dVe*/*Vp* values were calculated based on Eq. (2). Where $V_{PS(I)}$ and $V_{PS(I+1)}$ are the retention volumes of two successive PS standards with low and high molecular weights, respectively.

$$\left(\frac{dVe}{Vp}\right)_{(I)} = \frac{V_{PS(I)} - V_{PS(I+1)}}{V_{Toluene} - V_{2,000,000}} \quad (2)$$

The average pore size, $D_{p_{avg}}$ was determined based on the following expression. Where $(dVe/Vp)_{(I)}$ is the volume fraction of the pores with a diameter of $D_{p_{(I)}}$.

$$D_{p_{avg}} = \sum \left(\frac{dVe}{Vp}\right)_{(I)} \times D_{p_{(I)}} \quad (3)$$

The specific surface area of poly(GDMA-*co*-GDGDA) particles was determined by a surface area and pore size analyzer (Quantachrome, Nova 2200E, U.K.).

2.5. Synthesis of polymer samples for molecular weight determination

To use real polymer samples for molecular weight determination by aqueous size exclusion chromatography on the columns packed with the poly(GDMA-*co*-GDGDA) particles produced, two different polymers were synthesized by atom transfer radical polymerization (ATRP).

For the synthesis of a zwitterionic polymer, poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide), poly(MEDSPAH), CuBr (36 mg) and BiPy (78 mg) were dissolved in DDI water (5 mL) in a Schlenk tube. The monomer, MEDSPAH (10 mmol) was then dissolved in the medium. For deoxygenation, the sealed tube was evacuated by a vacuum pump for 5 min and the medium was purged with nitrogen for 10 min. The initiator, EBrIBu (0.10 mmol) was added and the polymerization was conducted at room temperature (24 °C) for 24 h under a nitrogen atmosphere. At the end of the polymerization, poly(MEDSPAH) was precipitated in MeOH. The dissolution in water and precipitation in methanol were repeated several times. Finally the polymer was dried in vacuo at 70 °C for 24 h.

A cationic polymer, poly(2-dimethylaminoethyl methacrylate), poly(DMAEM) was also obtained by ATRP. For this purpose, CuCl (5 mg), HMTETA (16 μL) and $CuCl_2$ (1 mg) were dissolved in anisole (2 mL) in a Schlenk tube. The monomer, DMAEM (1 mL) was then added. The sealed tube was evacuated by a vacuum pump for 5 min and purged with nitrogen for 10 min. The initiator, EBrIBu (20 μL) was added and polymerization medium was heated to 80 °C. The polymerization was conducted at 80 °C for 24 h under a nitrogen atmosphere. The polymerization medium was poured into excess diethylether and poly(DMAEM) was precipitated. The dissolution in anisole and precipitation in diethylether was repeated several times. Finally, the polymer was dried in vacuo at 70 °C for 24 h.

2.6. Aqueous size exclusion chromatography

Aqueous size exclusion chromatography experiments with dextran standards were performed in a semimicro and micro-HPLC system (Dionex, UMX-3000, U.S.A.) using distilled-deionized water as the mobile phase. In these runs, two stainless steel columns (100 mm × 1 mm i.d. and 150 mm × 2.0 mm i.d.) packed with poly(GDMA-*co*-GDGDA) particles synthesized with different seed latex to monomer ratios were used. The size-exclusion chromatograms were recorded by diode array detector (DAD) operated at 220 nm, at room temperature with the mobile phase flow rates ranging between 50 and 250 μL/min. The analytes used in aqueous SEC were fructose and dextran standards with average MWs between 700 and 670,000 Da. For SEC calibration curves obtained with dextran standards, the distribution coefficient (K_o) was determined by Eq. (4). Where $V_{D(I)}$ is the retention volume of selected dextran standard with distilled water as the mobile phase.

$$K_o = \frac{V_{D(I)} - V_{670,000}}{V_{fructose} - V_{670,000}} \quad (4)$$

The average molecular weights of three different polymers were also determined by aqueous size exclusion chromatography. As mentioned before, a zwitterionic polymer, poly(MEDSPAH) and a cationic polymer, poly(DMAEM) were synthesized by ATRP. A non-ionic polymer, poly(VA) with known molecular weight (molecular weight at peak maximum: 72 kDa, 100% hydrolysed, Merck A.G. Germany) was also used in the molecular weight measurements. The molecular weight at peak maximum for poly(VA) and poly(MEDSPAH) was determined at room temperature (22 °C) using dextran standards in a column 150 mm × 2 mm i.d. packed with poly(GDMA-*co*-GDGDA) particles obtained with the seed latex to monomer ratio of 0.038 g/mL. In these runs, the calibration curve for dextran standards was obtained using 0.001 N H_2SO_4 solution at a flow rate of 100 μL/min as mobile phase. 1 μL of sample polymer solution at a concentration of 10 mg polymer/mL DDI water was injected under the same chromatographic conditions. The chromatograms obtained with dextran standards and polymer samples were recorded at 195 nm. In the determination of molecular weight at peak maximum for poly(DMAEM), 0.001 N NaOH solution was used as the mobile phase both for dextran standards and poly(DMAEM) injections. The chromatograms for dextran standards and poly(DMAEM) were recorded at 200 nm. The other chromatographic conditions were identical with those used for the molecular weight determinations of Poly(VA) and poly(MEDSPAH).

3. Results and discussion

3.1. Size and morphological properties of particles

In this study, synthesis of hydrophilic, monodisperse macroporous particles was performed for use as a new and highly polar stationary medium in aqueous size exclusion chromatography. For this purpose, acrylic monomers with hydroxyl functionality, GDMA and GDGDA were selected. The molecular structures of selected monomers are given in Fig. 1. Note that both hydrophilic monomers were selected in the form of crosslinking agents. Among the selected structures, GDGDA is more polar with respect to GDMA since it contains three hydroxyl groups (Fig. 1) [17]. GDMA is less soluble in water than GDGDA and necessary to obtain sufficient rigidity in the resulting beads. The preliminary experiments showed that the synthesis of stable, rigid macroporous beads in spherical form was not possible by using only GDGDA as the monomer phase in a seeded polymerization using water as continuous medium. For this reason, GDMA was included in the polymerization mixture and the copolymer particles were

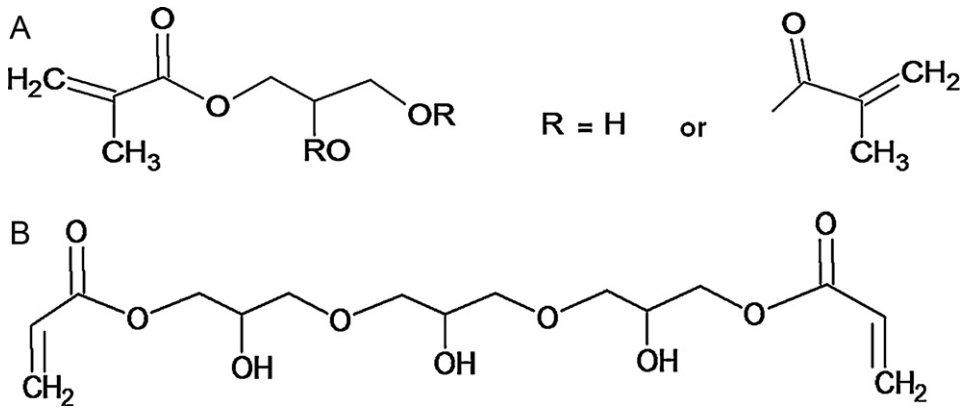

Fig. 1. The molecular structures of monomers used in the synthesis of monodisperse-porous particles (A) glycerol dimethacrylate (GDMA) and (B) glycerol-1,3-diglycerolate diacrylate (GDGDA).

synthesized using a monomer phase containing 50% (v/v) GDMA and 50% (v/v) GDGDA. The introduction of GDMA into the monomer phase could be also considered as a factor increasing the solubility of GDGDA in the monomer phase due to their similar structures. According to the authors' knowledge, no attempt has previously been made for the direct synthesis of rigid, monodisperse macroporous hydrogel beads as a stationary medium in aqueous size exclusion chromatography. Based on this reason, the present study was performed.

In this study, different types of seed latexes including poly(styrene), poly(methyl methacrylate) and poly(GMA) were tried for the synthesis of poly(GDMA-*co*-GDGDA) beads by seeded microsuspension polymerization. The preliminary experiments showed that polar monomer mixture containing GDMA and GDGDA was better absorbed by poly(GMA) seed particles. Poly(GDMA-*co*-GDGDA) particles were synthesized with different seed latex to monomer ratios. The seed latex to monomer ratio was selected as the variable since our previous studies demonstrated that this variable was one of the most effective polymerization conditions to regulate the average size and porosity characteristics of monodisperse macroporous particles synthesized by using acrylic monomers [18,19]. The electron micrographs of the poly(GDMA-*co*-GDGDA) particles prepared with different seed latex to monomer ratios are given in Fig. 2. As seen here, nearly monodisperse spherical particles with porous structure were obtained by the copolymerization of GDMA and GDGDA in the proposed seeded polymerization procedure. The average particle size and the coefficient of variation values calculated based on the SEM photos in Fig. 2 are given in Table 1. The SEM photos containing 70–120 beads were used for the determination of these values. As seen here, the average particle size decreased with increasing seed latex to monomer ratio. Higher seed latex to monomer ratio

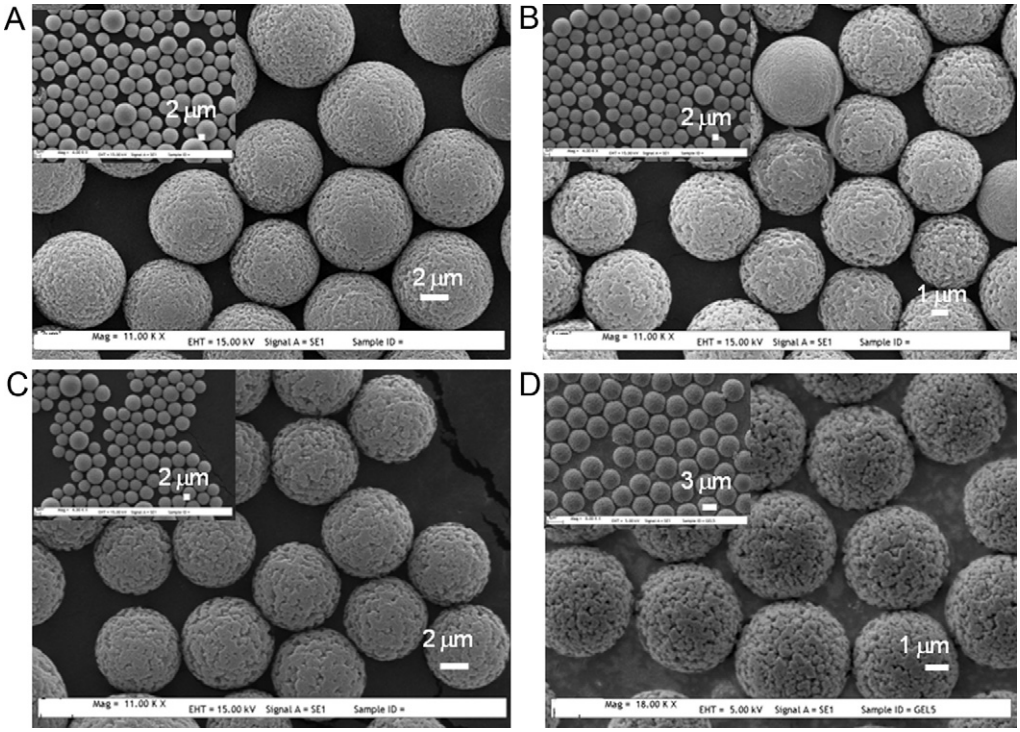

Fig. 2. Scanning electron micrographs of poly(GDMA-*co*-GDGDA) particles. Particles prepared with different seed latex to monomer ratios (g/mL); A: 0.038, B: 0.048, C: 0.058, D: 0.078. Magnifications: 4000× for A–C and 8000× for D, the bar represents 2 μm in A–C and 3 μm in D. Magnifications for insets: 11,000× for A–C and 18,000× for D, respectively. The bar represents 2 μm in A, C and 1 μm in B and D, respectively.

Table 1
The properties of poly(GDMA-*co*-GDGDA) particles synthesized for aqueous size exclusion chromatography.

SL/M (g/mL)	D_p [a] (μm)	CV[a] (%)	MPS[b] (nm)	SSA[c] (m^2/g)
0.038	6.74	8.1	26.2	20.78
0.048	6.18	9.0	21.0	18.13
0.058	5.72	5.2	30.8	15.64
0.078	4.50	4.0	44.3	17.48

SL/M, seed latex to monomer ratio; D_p, mean particle diameter; CV, coefficient of variation for size distribution; MPS, mean pore size; SSA, specific surface area.
[a] Determined by the evaluation of three separate SEM photos.
[b] Determined by inverse size exclusion chromatography.
[c] Determined by surface area and pore size analyzer.

resulted in poly(GDMA-*co*-GDGDA) particles with narrower size distribution (i.e. lower coefficient of variation for size distribution). In our previous study, seed latex to monomer ratio was changed in a wider range and the effects of polymerization conditions on the porosity properties of poly(GDMA-*co*-GDGDA) particles were investigated [17]. However, poly(GDMA-*co*-GDGDA) particles with significantly broader particle size distributions were obtained with the seed latex to monomer ratios lower than 0.038 g/mL [17]. On the other hand, the use of seed latex to monomer ratios higher than 0.078 g/mL gave poly(GDMA-*co*-GDGDA) particles with large, crater-like pores [17]. In this study, seed latex to monomer ratio was kept between 0.038 and 0.078 g/mL to obtain poly(GDMA-*co*-GDGDA) particles with suitable particle and pore size values for size exclusion chromatography.

3.2. Inverse-size exclusion chromatography

The porosity characteristics of the particles were determined by inverse size exclusion chromatography performed using THF as the mobile phase and toluene and PS standards with average molecular weights between 1000 and 2,000,000. The representative size exclusion chromatograms of PS standards are given in Fig. 3. As seen here, symmetrical peaks at different retention times were obtained for the PS standards with different average molecular weights. However, the peak for MW 770 seems to overlap with the MW 3680 and the peak for MW 2,000,000 seems to overlap with the MW 550,000. Different peak points were observed for the polystyrene standards with MWs between 3680 and 550,000 Da. The pore size distribution of packing material is not suitable for MW determination outside of this range. Note that,

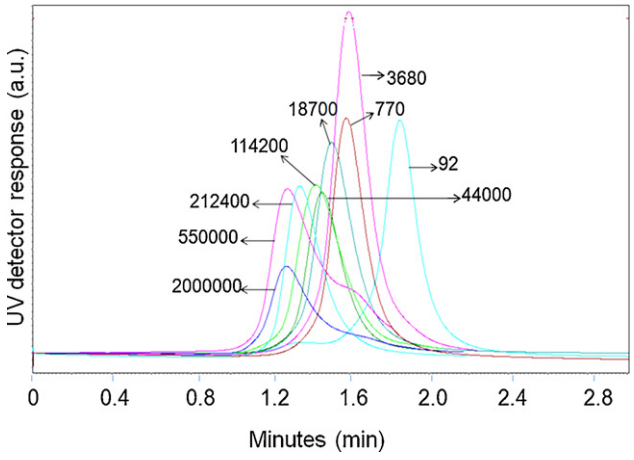

Fig. 3. Size exclusion chromatograms of polystyrene standards. Column: 50 mm × 7.8 mm i.d., mobile phase: THF, flow rate: 1 mL/min. UV detection at 254 nm. Polystyrene standards with average molecular weights 1000–2,000,000 Da. Concentration: 10 mg/mL. Injection volume: 20 μL. The average molecular weights (Da) of polystyrene standards are given in the chromatograms.

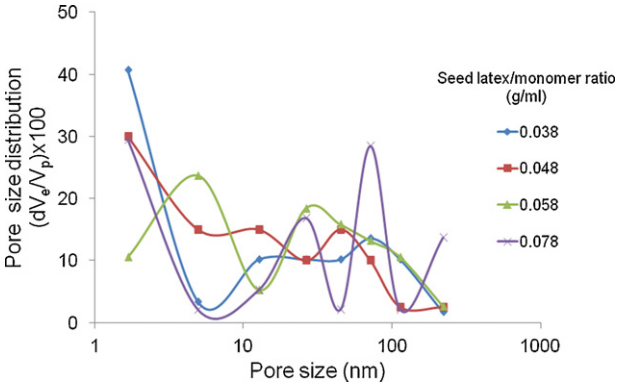

Fig. 4. Pore size distribution curves for poly(GDMA-*co*-GDGDA) particles.

although poly(GDMA-*co*-GDGDA) beads were designed as a potential stationary medium in aqueous size exclusion chromatography, they were also suitable for the size exclusion chromatography in organic media.

The pore size distribution curves obtained by inverse-size exclusion chromatography for the poly(GDMA-*co*-GDGDA) particles synthesized by different seed latex to monomer ratios are given in Fig. 4. As seen here, the pores were in the range of 2–120 nm for all particles. No appreciable change in the pore-size range was observed with the seed latex to monomer ratio. The average pore-size values determined by inverse size exclusion chromatography and the specific surface areas determined by BET are included in Table 1 for the poly(GDMA-*co*-GDGDA) particles synthesized with different seed latex to monomer ratios. As seen here, the average pore size decreased, and the specific surface area increased with decreasing seed latex to monomer ratio. This behavior is consistent with the results of our previous studies on the synthesis of acrylic based, monodisperse macroporous particles [18,19]. As explained previously, the viscosity of porogen mixture increases with increasing seed latex to monomer ratio and this leads to the formation of larger agglomerates during the fixation of crosslinked nuclei within the forming macroporous-particles [2,18,19]. Hence larger pores were obtained within a crosslinked structure containing larger agglomerates.

The SEC calibration curves obtained using THF as the mobile phase with PS standards for the columns packed with the poly(GDMA-*co*-GDGDA) particles synthesized with different seed latex to monomer ratios are given in Fig. 5. As seen here, poly(GDMA-*co*-GDGDA) particles synthesized with different seed

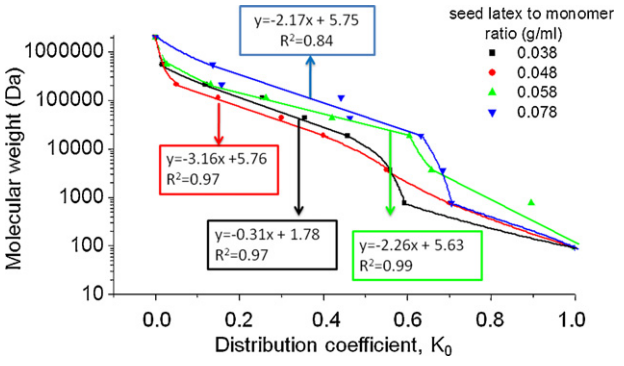

Fig. 5. Size exclusion chromatography calibration curves for polystyrene standards. Column packed with poly(GDMA-*co*-GDGDA) particles prepared using different seed latex to monomer ratios. Column: 50 mm × 7.8 mm. Mobile phase: THF, flow rate: 1 mL/min. UV detection at 254 nm. Polystyrene standards MW: 770, 3680, 18,700, 44,000, 114,200, 212,400, 550,000 and 2,000,000 Da. Analyte concentration: 5 mg/mL. Injection volume: 20 μL.

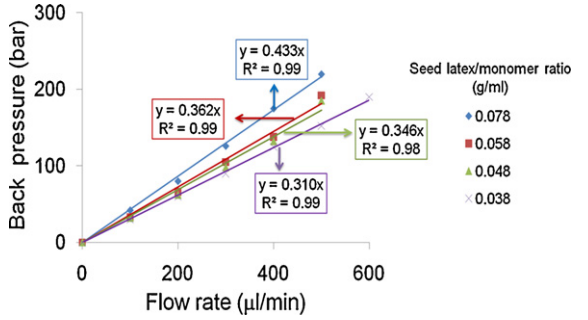

Fig. 6. Back-pressure at different flow rates. Column dimensions: 150 mm × 2.0 mm i.d. packed with poly(GDMA-*co*-GDGDA) particles prepared using different seed latex to monomer ratios. Mobile phase: distilled water.

latex to monomer ratios gave calibration curves exhibiting linearity for K_o values between 0.1 and 0.5. These packings seem suitable for molecular weight determination between 5×10^3 and 1×10^6 in the polar organic medium.

3.3. Aqueous size exclusion chromatography

Poly(GDMA-*co*-GDGDA) particles were slurry packed in semimicro and micro-HPLC columns and these columns were used as separation media for aqueous SEC. The back-pressure flow rate relationships of the columns packed with the poly(GDMA-*co*-GDGDA) particles are given in Fig. 6. As seen from the correlation coefficients close to unity, back pressure almost linearly increased with increasing flow rate for the packings synthesized with different seed latex to monomer ratios (Fig. 6). On the other hand, back-pressure at constant flow rate increased with increasing seed latex to monomer ratio. This behavior could be explained by increasing column porosity with decreasing seed latex to monomer ratio.

Size exclusion chromatograms obtained with dextran standards by using DDI water as the mobile phase in microbore (1 mm × 100 mm) and semimicro-HPLC (2 mm × 200 mm) columns packed with poly(GDMA-*co*-GDGDA) particles are given in Figs. 7 and 8, respectively. In these figures, the chromatograms obtained with different mobile phase flow rates are included. Dextran standards were eluted in less than 2 min in micro and semi-micro columns packed with poly(GDMA-*co*-GDGDA) beads obtained with the seed latex to monomer ratio of 0.038 g/mL.

The SEC calibration curves of microbore and semimicro-HPLC columns packed with the poly(GDMA-*co*-GDGDA) particles synthesized with different seed latex to monomer ratios are given in Fig. 9. As seen here, the curves obtained with the packings produced with different seed latex to monomer ratios are suitable for MW determination in the range of 5×10^3–5×10^5 Da in the aqueous medium in both columns. Only the curve obtained with the packing produced with the seed latex to monomer ratio of 0.078 in 1 mm i.d. column exhibited an earlier bending point at lower K_o. This behavior could be probably explained by the larger average pore size of this packing.

For molecular weight determination of real polymer samples, three different types of water soluble polymers were used. For this purpose, an ionic polymer, poly(DMAEM) and a zwitterionic polymer, poly(MEDSPAH) were synthesized by atom transfer radical polymerization. A non-ionic polymer, poly(VA) was obtained from a supplier. The molecular structures of the polymers selected for molecular weight determination are given in Fig. 10. The average molecular weights of poly(VA) and poly(MEDPSAH) were determined using the same mobile phase (i.e. 0.001 N aqueous H_2SO_4 solution) with a flow rate of 100 µL/min, in a semi-micro column (150 mm × 2 mm i.d.) packed with the poly(GDMA-*co*-GDGDA) beads obtained with the seed latex to monomer ratio of 0.038 g/mL

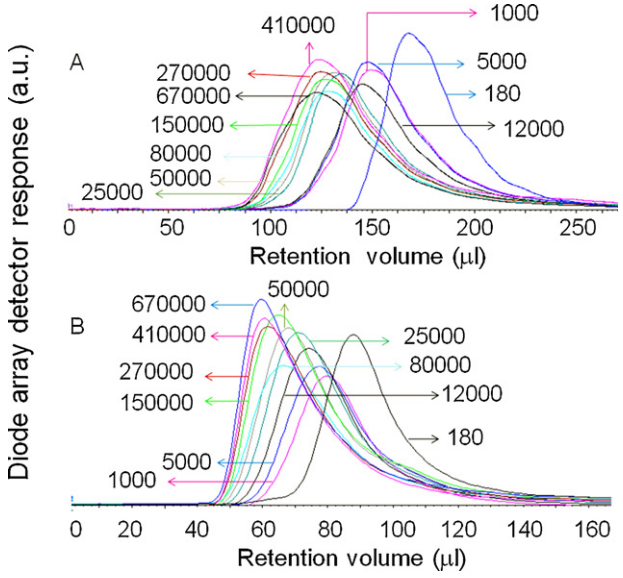

Fig. 7. Size exclusion chromatograms of dextran standards. Column: 100 mm × 1 mm i.d., mobile phase: distilled water. Seed latex to monomer ratio (g/mL): (A) 0.078, (B) 0.038. Mobile phase flow rate (µL/min): (A) 25, (B) 100. Injection volume: 0.5 µL. Analyte concentration: 10 mg/mL. Detection: DAD at 195 nm. The average molecular weights (Da) of dextran standards are given in the chromatograms.

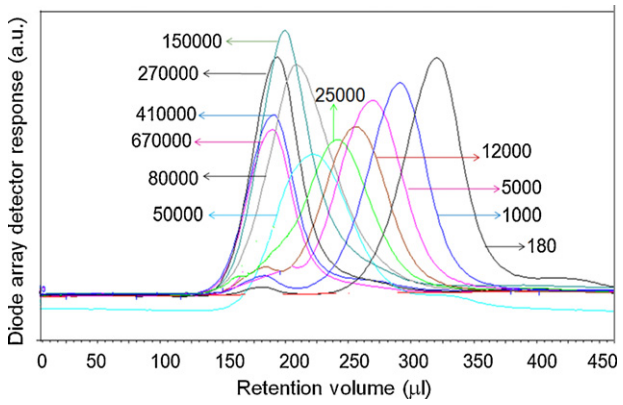

Fig. 8. Size exclusion chromatograms of dextran standards. Column: 150 mm × 2 mm i.d., mobile phase: distilled water. Seed latex to monomer ratio: 0.038 g/mL, mobile phase flow rate: 250 µL/min, injection volume: 0.5 µL, analyte concentration: 10 mg/mL, detection: DAD at 195 nm. The average molecular weights (Da) of dextran standards are shown in the chromatograms.

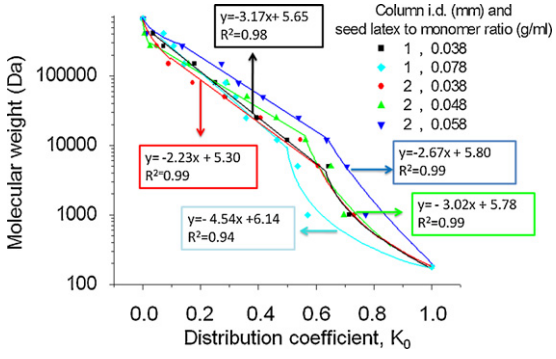

Fig. 9. SEC calibration curves for dextran standards in microbore and semi-micro-HPLC columns packed with the poly(GDMA-*co*-GDGDA) particles synthesized with different seed latex to monomer ratios. Dextran standards MW: 1000, 5000, 12,000, 25,000, 50,000, 80,000, 150,000 and 270,000, 410,000, 670,000 Da. The chromatographic conditions are given in Figs. 7 and 8.

Table 2
The molecular weights at peak points determined for the real polymer samples by the aqueous SEC performed on poly(GDMA-*co*-GDGDA) column.

Polymer	Synthesis	Mobile phase	Detection	Mp (Da)
Poly(VA)	–	0.001 N H_2SO_4	DAD, 190 nm	7.8×10^4
Poly(MEDSPAH)	ATRP	0.001 N H_2SO_4	DAD, 190 nm	4.9×10^4
Poly(DMAEM)	ATRP	0.001 N NaOH	DAD, 205 nm	2.9×10^4

Column 150 mm × 2.0 mm i.d. 100 μL/min, poly(GDMA-*co*-GDGDA) beads produced with seed latex to monomer ratio of 0.038 g/mL.

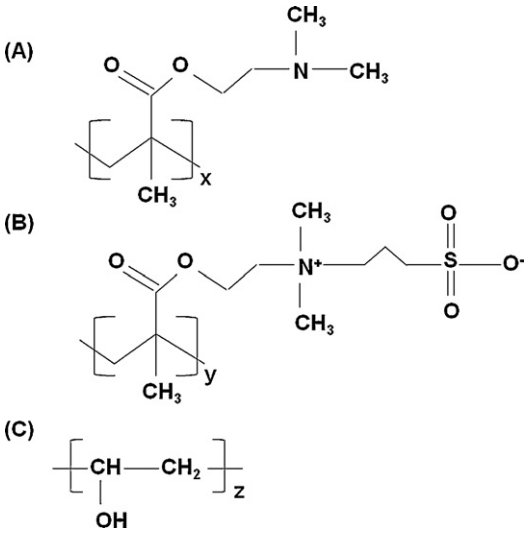

Fig. 10. Molecular structures of polymer samples selected for molecular weight determination (A) poly(2-dimethylaminoethyl methacrylate), poly(DMAEM), (B) poly([2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide), poly(MEDSPAH), and (C) poly(vinyl alcohol), poly(VA).

(Table 2). The size exclusion chromatograms obtained for poly(VA) and poly(MEDPSAH) are given in Fig. 11A together with the chromatograms of some dextran standards with different molecular weights. The average molecular weight of poly(DMAEM) was determined using another mobile phase (i.e. 0.001 N aqueous NaOH solution) by using the same column and under the chromatographic conditions (Table 2). The size exclusion chromatograms obtained for poly(DMAEM) and some dextran standards with different molecular weights are given in Fig. 11B. The molecular weight corresponding to the peak-maximum of SEC chromatogram was calculated using the calibration curve obtained with the dextran standards injected using the related mobile phase (i.e. 0.001 N H_2SO_4, 0.001 N NaOH) (Fig. 11C) [21]. The molecular weights of real polymer samples at the peak-maxima are given in Table 2. By the supplier, the molecular weight of poly(VA) at peak-maximum was given as 7.2×10^4. This value was found as 7.8×10^4 by the aqueous SEC performed in our study. The molecular weight at the peak point for poly(DMAEM) was in the same order of magnitude of average molecular weights of poly(DMAEM) samples synthesized via ATRP by different researchers [22,23]. These results indicated that poly(GDMA-*co*-GDGDA) beads were successfully tried as stationary medium in the molecular weight determination of real polymer samples by aqueous SEC.

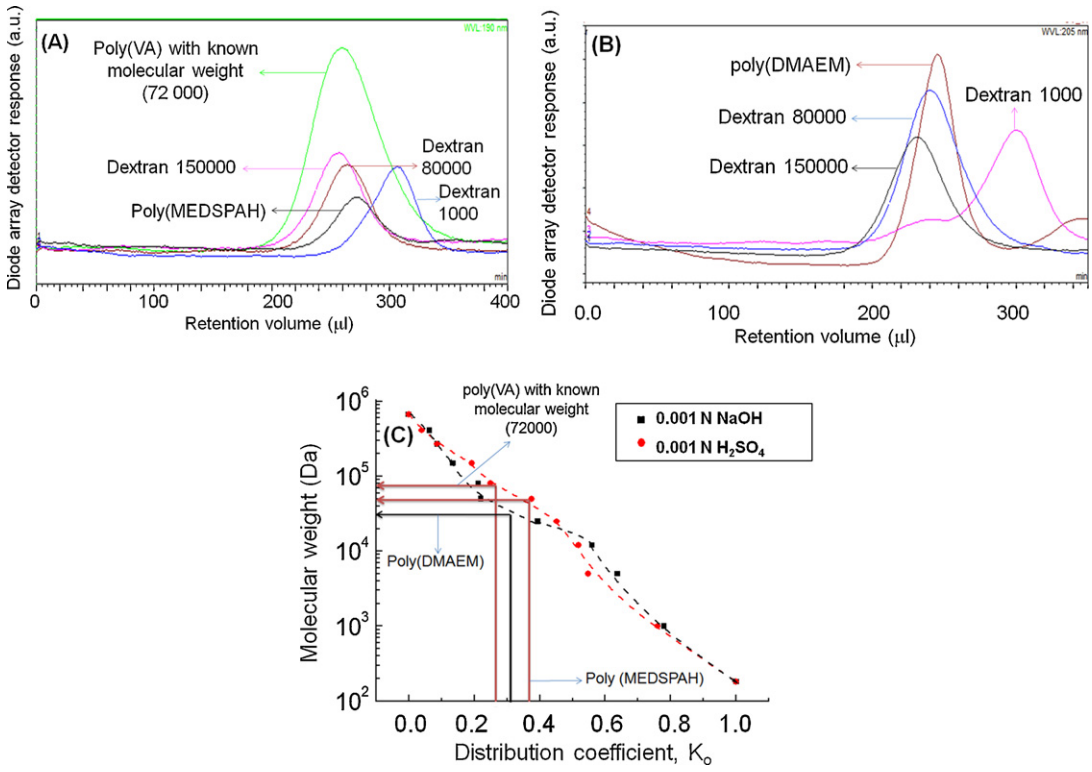

Fig. 11. Size exclusion chromatograms. (A) poly(MEDSPAH), poly(VA) and dextran standards (MW: 1000, 80,000 and 150,000 Da), mobile phase: 0.001 N H_2SO_4, detection: DAD at 190 nm, flow rate: 100 μL/min, injection volume: 1.0 μL, analyte concentration: 10 mg/mL, (B) poly(DMAEM) and dextran standards (MW: 1000, 80,000 and 150,000 Da), mobile phase: 0.001 N NaOH, detection: DAD at 205 nm, flow rate: 100 μL/min, injection volume: 1.0 μL, analyte concentration: 10 mg/mL, and (C) SEC calibration curves obtained with dextran standards under conditions given in (A) and (B). The average molecular weights (Da) of dextran standards are shown in the chromatograms.

4. Conclusion

Monodisperse porous poly(GDMA-*co*-GDGDA) beads with a highly hydrophilic nature due to their hydroxyl functionality were obtained in the size range of 4.5–6.7 μm with different porous properties. The beads were slurry packed in microbore and semimicro-HPLC columns and successfully used as a stationary phase in aqueous size exclusion chromatography mode in micro-liquid chromatography. The aqueous SEC runs were performed by using dextran standards in the molecular weight range of 1000 and 670,000 Da, by using mobile phase flow rates in the range of 25–250 μL/min. The results indicated that the newly synthesized packings were suitable for molecular weight determinations in the range of 5×10^3–5×10^5 in aqueous medium. The average molecular weights of different water-soluble polymers in ionic, non-ionic and zwitterionic forms were determined using a semi-micro-HPLC column packed with poly(GDMA-*co*-GDGDA) beads with very short analysis times. The micro and semi-micro columns packed with poly(GDMA-*co*-GDGDA) beads are promising tools for fast aqueous SEC of water soluble polymers.

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