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Nucleotide adsorption–desorption behaviour of boronic acid functionalized uniform-porous particles

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Abstract

In this study, nucleotide adsorption–desorption behaviour of boronic acid-carrying uniform, porous particles was investigated. The particles were produced by a ''multi-step microsuspension polymerization'' in the form of poly(styrene–vinylphenyl boronic acid–divinylbenzene) terpolymer. In the first step of the production method, uniform polystyrene latex particles (6.2 μm in size) were obtained by dispersion polymerization. These particles were first swollen by a low molecular mass organic agent (i.e. dibutylphthalate, DBP) and then by a monomer mixture including styrene (S), 4-vinylphenyl boronic acid (VPBA) and divinylbenzene (DVB). The particle uniformity was protected in both swelling stages by adjusting DBP/polystyrene latex and monomer mixture/polystyrene latex ratios. Polymerization of the monomer mixture in the swollen seed particles provided boronic acid-carrying uniform, porous particles 11–12 μm in size. To have uniform particles with different porosities and boronic acid contents, the feed concentration of boronic acid-carrying monomer and the monomer/seed latex ratio were changed. The particles were tried as sorbent for the adsorption of a model nucleotide (i.e., β-nicotinamide adenine dinucleotide, β-NAD). In the β-NAD adsorption experiments, the maximum equilibrium adsorption was obtained at pH 8.5 which was very close to pK_a of boronic acid. The incorporation of boronic acid functionality provided a significant increase in the β-NAD adsorption. In contrast to plain poly(styrene-*co*-divinylbenzene) particles, four-fold higher β-NAD adsorption was obtained with the boronic acid functionalized particles. β-NAD was desorbed from the particles with the yields higher than 90% by weight.

Keywords: Porous particles; Adsorption–desorption behaviour; Nucleotides; Boronic acid

1. Introduction

The uniform latex particles utilized as sorbents in bioaffinity chromatography have been usually obtained by using modified emulsion or dispersion polymerization techniques allowing the introduction of desired functionalities onto the particle surface. By following these production methods, the uniform latex particles carrying different functional groups like carboxyl [1–5], hydroxyl [6–9], amine [10–15] or chloromethyl [16–20] were obtained. For the chromatographic applications, the size and the morphological properties and the surface chemistry of latex particles should be appropriate for intended use. On the other hand, uniform-macroporous particles produced in micron-size range (especially in

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the size range of 5–20 μm) are reasonably promising materials as ''new-generation supports'' in the size exclusion chromatography (SEC) applications [21–32]. More regular flow regime in the chromatographic column originated from the uniform character of the support material usually provide chromatograms with higher resolution relative to those obtained with the conventional support materials prepared in the polydisperse form [21,26–28]. The uniform macroporous particles have been usually produced by the multi-stage seeded polymerization methods developed by starting from the mid 1980s [21]. Although these materials were obtained with different functionalities, no attempt has been made for the synthesis of uniform-chromatographic packing materials carrying boronic acid moiety.

The chromatographic applications involving the use of boronic acid functionalized support materials are collected under the title of ''boronate affinity chromatography''. These applications were performed based on the complex formation between boronate groups of support material and diol groups of the target molecules [33–41]. In the conventional boronate affinity chromatography applications involving the selective isolation of biomolecules like nucleotides, RNA, glycated proteins and glycoenzymes, a single column containing boronic acid-carrying particles was utilized. For this purpose, boronic acid functionalized-polyacrylamide, agarose and polyacrylate based gels were commonly preferred as support materials [33–41]. The sample including target biomolecules was loaded into the column and diol-carrying biomolecules were selectively adsorbed onto the column material by the complex formation. The adsorbed molecules were eluted by applying a mobile phase including appropriate desorption agents.

Various affinity-HPLC applications using boronate functionalized-column materials were developed for the separation of glycated proteins. A high-performance affinity chromatography system was constructed by using serially connected columns. This system included an anion-exchange column to separate total albumin and a consecutive boronate functionalized-column to separate glycated and nonglycated albumin [42,43]. The boronate functionalized column was obtained by the covalent attachment of *m*-aminophenyl boronic acid onto a conventional high-performance anion-exchange column [42,43]. In the chromatographic studies similar to this application, boronate affinity column was first used for the enrichment of glycoproteins in the serum and the enriched mixture was then applied to a high-performance anion-exchange column in the HPLC system [44–47]. In a typical ''affinity-HPLC'' application developed by Koyama and Terauchi, a column including boronic acid functionalized-porous particles with diameter of 14 μm was used for the quantification of glycated serum proteins [48]. It should be emphasized that the single separation medium in the proposed HPLC system was the porous-boronate functionalized particles. In the application developed by Hjerten and Li, a column including *m*-aminophenylboronic acid attached-nonporous agarose beads with diameters of 12–15 μm was also used as a single stationary phase in a HPLC system constructed for the fractionation of glycosylated from non-glycosylated haemoglobin [49].

All these chromatographic applications involve the presence of boronic acid groups on the support material having an interaction capability with the diol groups of target biomolecules. In our study, boronic acid functionalized uniform-macroporous particles were obtained by a multistep microsuspension polymerization, by including a boronic acid-carrying monomer in the polymerization recipe (4-vinylphenylboronic acid, VPBA). The average size, porosity properties and surface functional groups of the particles synthesized in our study were reasonably similar to those synthesized by Koyama and Terauchi [48]. The particles obtained in our study were proposed as potential column materials for ''affinity-HPLC applications'' since the similar particles exhibited a good chromatographic performance in the separation of glycated proteins by the same method [48]. After production and characterization stages, the description of reversible adsorption–desorption behaviour of target molecules is essential in the development of stationary phases for ''affinity-HPLC applications''. For this purpose, β-nicotinamide adenine dinucleotide (β-NAD) was selected as a model target molecule carrying diol groups and the interaction between selected molecule and proposed material was investigated in batch fashion.

2. Experimental

2.1. Materials

Styrene (Yarpet AS, Turkey) was distilled under vacuum and stored in the refrigerator. The cross-linking agent, divinylbenzene (DVB, 55% para and meta-divinylbenzene isomers, Aldrich Chem. Co., USA) was extracted with 5% (w/w) aqueous NaOH solution for the removal of inhibitor from the isomer mixture. 4-vinylphenylboronic acid (VPBA, Aldrich Chem. Co., Milwaukee, WI, USA) was selected as the reactive comonomer-carrying boronic acid functionality and utilized without further purification. Sodium dodecyl sulfate (SDS, Sigma Chem. Co., St. Louis, MO, USA) was the emulsifier in the preparation of the emulsion media. Dibutyl phthtalate (DBP, Polisan AS, Turkey) was selected as the diluent. An oil soluble initiator, benzoyl peroxide (BPO, Aldrich Chem. Co.) was used for the repolymerization of styrene–divinylbenzene–4-vinylphenylboronic acid mixture in the swollen seed particles. β-nicotinamide adenine dinucleotide (β-NAD, Cat No:N1511, Sigma Chemical Co.) was used as the model nucleotide. The buffer solutions for the β-NAD adsorption experiments were prepared by using *N*-[2-hydroxyethyl]piperazine-*N′*-[2-ethane sulfonic acid] (HEPES, Sigma Chem. Co.). Distilled–deionized water was used in the preparation of the media for polymerization and all adsorption experiments.

2.2. Preparation of uniform polystyrene seed particles

A detailed procedure for the synthesis of uniform polystyrene (PS) seed particles 6.2 μm in size was described elsewhere [31].

2.3. Preparation of uniform poly(styrene–vinylphenylboronic acid–divinylbenzene) particles

Boronic acid functionalized uniform particles were obtained by a polymerization method termed as ''multi-step microsuspension polymerization''. Typically, DBP (0.175 ml) was emulsified in aqueous medium (20 ml) including 0.25% (w/w) sodium dodecyl sulfate (SDS). The emulsion was obtained by the sonication of the medium including DBP and SDS solution in an ultrasonic water bath (Bransonic 200, USA) for about 1 h. An aqueous dispersion (approximately 2.0 ml) including polystyrene (PS) seed particles (0.175 g) was added into the DBP emulsion. Final dispersion was stirred at room temperature for 24 h at 400 rpm for the absorption of DBP by the PS seed particles. In the next step, a monomer phase including S (0.5 ml), DVB (0.5 ml), VPBA (0.1 g) and BPO (0.06 g), was emulsified in an aqueous solution (18 ml) including 0.25% (w/w) SDS, by sonication. Monomer emulsion was combined with the aqueous dispersion of 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. The emulsion was purged with bubbling nitrogen for 5 min. Polymerization of monomer phase within the swollen seed particles was performed at 70 °C for 24 h at 120 cpm shaking rate in a sealed pyrex reactor. Repolymerization stage led to the uniform poly(styrene–4-vinylphenylboronic acid–divinylbenzene), poly(S–VPBA–DVB) particles. To remove the diluent and by products, the particles were cleaned with ethanol extensively, by performing successive centrifugation and decantation steps [31]. Finally, the particles were washed with water and redispersed in distilled–deionized water.

2.4. Characterization of uniform-macroporous poly(S–VPBA–DVB) particles

The size and size distribution of poly(S–VPBA–DVB) particles were determined by a scanning electron microscope as described elsewhere [28,31]. An aqueous dispersion of cleaned latex particles (approximately 0.1 ml) was spread onto a copper disk and water was evaporated at room temperature. Dried particles were coated with a thin layer of gold (approximately 100 Å) in a sputter coater (BioRad Instruments, CA, USA). The specimens were examined and photographed in a Scanning Electron Microscope (JEOL, JEM 1200 EX, Tokyo, Japan). The particle interiors were examined by transmission electron microscopy [28,31]. For this purpose, dried particles (100–200 mg) were fixed in 1% (w/w) aqueous osmium tetraoxide (OsO_4^-) solution (Sigma

Chemical Co.) and dehydrated in a graded series of alcohols, then embedded in Araldite CY 212 resin (Agar Scientific, Essex, UK). Thin sections were cut serially (60–90 nm) by ultratom (LKB, Sweden), mounted on 100 mesh grids (Agar Scientific) and examined under a transmission electron microscope (JEOL, JEM 1200 EX, Tokyo, Japan). Bulk and surface structures of poly(S–VPBA–DVB) particles were analyzed by FTIR and FTIR-DRS (Fourier Transform Infrared-Diffuse Reflectance Spectroscopy). FTIR and FTIR-DRS spectra were obtained using KBr tablets and KBr powder, respectively. The particles and KBr were first dried in vacuum at 50 °C for 4 days and over $CaCl_2$ in vacuum for 3 days. The mass–charge density of the particles was determined by potentiometric titration. For this purpose, poly(S–VPBA–DVB) particles were subsequently reacted with aqueous KOH solution (25 ml, 0.05 N). The particles were separated by centrifugation and the unreacted KOH in the supernatant was determined by potentiometric titration with 0.1 N standard HCl solution. The mass charge density expressed in equivalents of surface acid groups per unit weight of particles (Q, meq/g) was calculated according to Eq. (1):

$$Q = V_b \times (C_i - C_f)/W \quad (1)$$

where C_i and C_f (mM) are the initial and final base (i.e. KOH) concentrations determined by potentiometric titration with 0.1 N HCl solution. V_b (ml) is the volume of base solution interacted with poly(S–VPBA–DVB) particles and W (g) is the dry weight of poly(S–VPBA–DVB) particles.

2.5. β-NAD adsorption experiments

A certain amount of poly(S–VPBA–DVB) particles (0.12 g) were dispersed in a HEPES buffer solution (20 ml, pH:8.5, +4 °C) including β-NAD (1 mg/ml) and $MgCl_2$ (50 mM). β-NAD adsorption experiments were conducted +4 °C for 2 h with 250 rpm stirring rate. For the derivation of adsorption isotherm, initial β-NAD concentration was changed between 0.04 and 1.40 mg/ml. At the end of the adsorption period, the entire mixture was centrifuged at 4000 rpm for 5 min and the supernatant was separated. Final β-NAD concentration in the supernatant was determined by measuring the absorbance (A_f) in a UV–Vis Spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 260 nm [36–38]. β-NAD adsorbed (i.e. equilibrium β-NAD concentration on the particles, $Q_{\beta\text{-NAD}}$) was calculated according to the following expression. Where $C_{o(\beta\text{-NAD})}$ and $C_{f\,(\beta\text{-NAD})}$ (mg/ml) are the initial β-NAD concentration in HEPES buffer and β-NAD concentration in the supernatant, respectively. V (ml) and M_p (g) are the volume of buffer solution, and the amount of poly(S–VPBA–DVB) particles, respectively.

$$Q_{\beta\text{-NAD}} = [(C_{o(\beta\text{-NAD})} - C_{f\,(\beta\text{-NAD})})/C_{o\,(\beta\text{-NAD})}] \times V/M_p \quad (2)$$

2.6. β-NAD desorption experiments

A medium defined in the literature was selected for the desorption of β-NAD from the poly(S–VPBA–DVB) particles [35–37]. β-NAD adsorbed particles loaded with different β-NAD initial concentrations were placed in this medium (i.e., 20 ml, 0.05 M borate buffer including 0.1 M NaCl at +4 °C) at pH:10.2. The dispersion was stirred for 1 h at +4 °C at 300 rpm. The final absorbance was measured at 260 nm. Desorption ratio was calculated as the ratio of desorbed amount of β-NAD (mg) to the adsorbed amount of β-NAD on the poly(S–VPBA–DVB) particles (mg).

3. Results and discussion

In this study, boronic acid-carrying uniform macroporous particles were synthesized as potential ‘‘affinity-HPLC support’’ by ‘‘multistep microsuspension polymerization’’. The presented material has two significant advantages relative to the ‘‘affinity-HPLC supports’’ proposed previously.

(i) The introduction of boronic acid functionality onto the conventional support materials involves an activation step [33–41]. In this stage, the functional groups on the support materials (i.e., hydroxyl, amine or carboxyl) were reacted with proper agents (i.e., cyanogen bromide, a water soluble carbodiimide or 1,4-butanediol diglycidyl ether, etc.) for the generation of active sites allowing the covalent attachment of boronic acid-carrying ligands (like *m*-aminophenyl boronic acid, APBA). After production of plain particles, particularly the activation and the ligand attachment are additional and time-consuming processes involving the use of toxic reagents. In our case, boronic acid functionality was directly incorporated onto the macroporous particles during the polymerization by using a boronic acid-carrying monomer.

(ii) All boronic acid-carrying particles previously tried in the affinity-HPLC applications were produced in the polydisperse form [42–49]. In our study, the particles with boronic acid functionality were synthesized in the uniformly-sized form. As ‘‘new generation supports’’ in chromatographic applications, uniform particles provide liquid chromatograms with higher resolution relative to the polydisperse packing materials, due to more regular flow regime in the column [21,26–28].

For the developed support material, the synthesis, characterization and reversible adsorption–desorption behaviour of a model diol-carrying molecule (β-NAD) are given below.

3.1. Characterization of uniform poly(S–VPBA–DVB) particles

Various polymerization recipes were used for the synthesis of poly(S–VPBA–DVB) particles with different properties. The production conditions and the properties of particles are listed in Table 1. As seen here, two sets of experiments were designed. In the first set, the feed concentration of VPBA was changed between 0 and 5 mg/ml by fixing (S–DVB)/seed latex ratio at 9.14 ml/g. Here, DBP/seed latex ratio was 1 ml/g. The experiment encoded as BA1 provided poly(S–DVB) particles with relatively wider size distribution. The introduction of VPBA into the polymerization led to the final particles with narrower size distribution (i.e. experiments encoded as BA2 and BA3). The electron micrographs showing the surface morphology of particles obtained in the first set are given in Fig. 1. As seen here, both poly(S–DVB) and poly(S–VPBA–DVB) particles produced with two different VPBA feed concentrations had a macroporous surface. However, larger pores were observed on the surface of the particles produced in the absence of VPBA (Fig. 1A). The introduction of VPBA provided particles with lower surface porosity. The surface of VPBA-carrying particles produced with two different VPBA feed concentrations (2.5 and 5 mg/ml) had reasonably smaller pores. To show the porous character of the surface more clearly, a sample electron micrograph of the particles obtained with the VPBA feed concentration of 2.5 mg/ml was taken with higher magnification and given in Fig. 2.

In our polymerization method, the porogen solution comprised of two components which were the low molecular mass organic agent (i.e. DBP) and the linear polystyrene coming from the seed latex. In the second set, the particles having higher bulk and surface porosities were aimed. For this purpose,

Table 1
The production conditions and properties of boronic acid-carrying uniform macroporous particles

Code	Seed latex (g)	DBP (ml)	Styrene (ml)	DVB (ml)	VPBA (g)	D_n (μm)	D_w (μm)	C.V. (%)	Mass–charge (meq/g) density
BA1	0.175	0.175	0.80	0.80	0	11.85	12.21	12.8	0.66
BA2	0.175	0.175	0.80	0.80	0.1	12.15	12.30	7.03	0.76
BA3	0.175	0.175	0.80	0.80	0.2	12.29	12.42	5.95	0.80
BA4	0.175	0.175	0.50	0.50	0	11.60	11.67	4.20	0.82
BA5	0.175	0.175	0.50	0.50	0.1	11.27	11.34	4.74	1.09
BA6	0.175	0.175	0.50	0.50	0.2	11.11	11.23	6.40	1.16

DBP, Dibutylphthalate; DVB, Divinylbenzene; VPBA, 4-Vinylphenylboronic acid; D_n, Number-average diameter (μm); D_w, Weight-average diameter (μm); C.V., Coefficient of variaton. Common conditions: SDS concentration, 0.25% (w/w); BPO concentration, 60 mg/ml; Polymerization temperature, 70 °C; Shaking rate, 120 cpm; Time, 24 h.

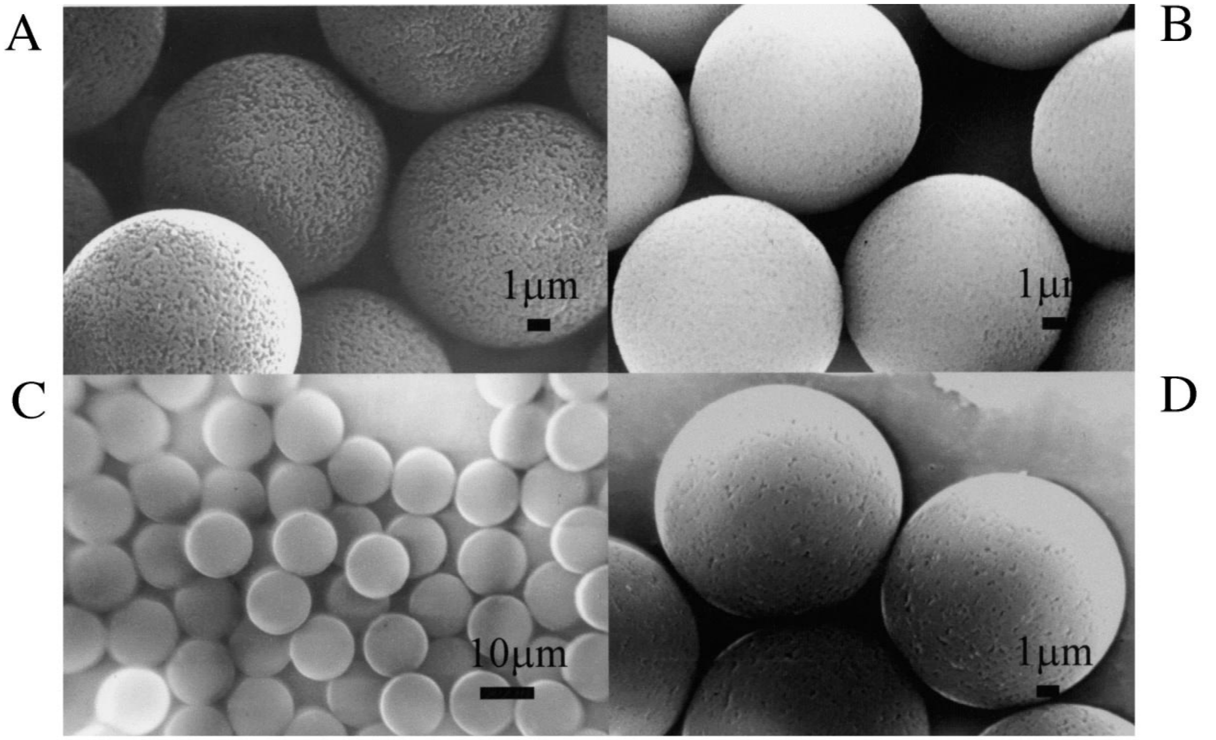

Fig. 1. Scanning electron micrographs of uniform-macroporous poly(S–VPBA–DVB) particles, (S–DVB)/seed latex ratio: 9.14 ml/g, VPBA feed concentration (mg/ml): (A) 0, Mag: ×4000; (B) 2.5, Mag: ×4000; (C) 5.0, Mag: ×1000; (D) 5.0, Mag: ×4000.

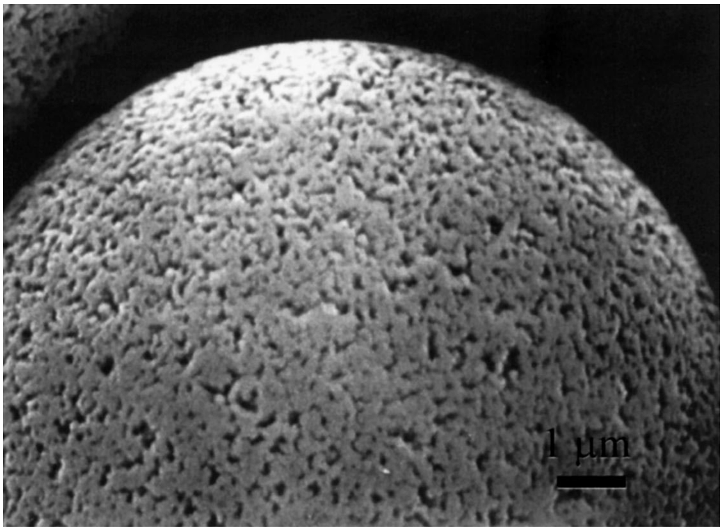

Fig. 2. Scanning electron micrograph showing the detailed surface morphology of poly(S–VPBA–DVB) particles with fine porous structure obtained with the (S–DVB)/seed latex ratio of 9.14 ml/g and VPBA feed concentration of 2.5 mg/ml. Mag: ×10K.

S–DVB/seed latex ratio was decreased to 5.71 ml/g (Table 1). By this modification, the polymeric part of porogen was increased with respect to the volume of S–DVB mixture used in the repolymerization. In other words, more porogen was utilized for per unit mass of porous particles. In this set, the feed concentration of VPBA was also changed between 0 and 5 mg/ml. The properties of the particles produced in the second set are also given in Table 1. As seen here, these particles had narrower size distribution (i.e. lower C.V. values) relative to those produced in the first set since they were obtained with a lower monomer phase/seed latex ratio [28,31]. The SEM photographs of the produced particles are given in Fig. 3. These photographs clearly indicated that the surface porosity was higher

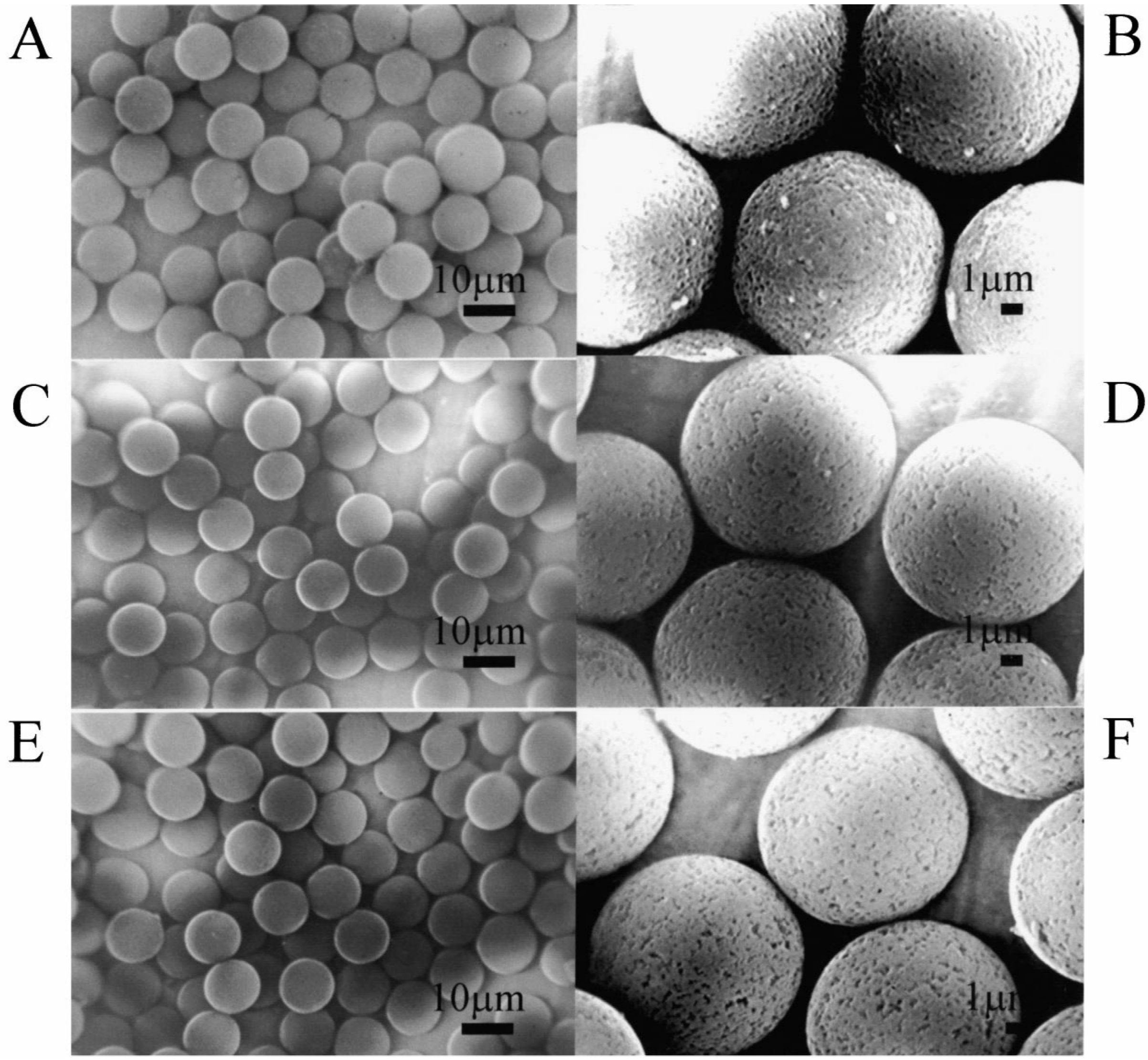

Fig. 3. Scanning electron micrographs of uniform-macroporous poly(S–VPBA–DVB) particles, (S–DVB)/seed latex ratio: 5.71 ml/g, VPBA feed concentration (mg/ml): (A) 0, Mag: ×1000; (B) 0, Mag: ×4000; (C) 2.5, Mag: ×1000; (D) 2.5, Mag: ×4000; (E) 5.0, Mag: ×1000; (F) 5.0, Mag: ×4000.

relative to those of the first set particularly for the particles produced in the presence of VPBA. Relatively larger pores were observed on the surface of the particles produced with the VPBA feed concentrations of 2.5 and 5 mg/ml. For both sets, the internal structures of particles produced with the VPBA feed concentration of 5 mg/ml were compared in Fig. 4. As seen here, both porosity and pore number density were relatively low in the cross-section of the particles produced by using the first-set conditions (Fig. 4A). However, an internal-structure including pores regularly distributed in the whole cross-section was obtained for the particles produced by the modified conditions (i.e. the second set in Table 1). Either the number density of pores or the porosity were higher for this case (Fig. 4B).

A commonly accepted mechanism for the pore formation process in the uniform latex particles was proposed by Cheng et al. [24,25]. According to this mechanism, the first stage in the pore formation process is described as the production and agglomeration of low-energy and highly crosslinked microspheres by phase separation taking place between the crosslinked polymer phase and the porogen solution including linear polystyrene coming from seed latex and low molecular mass organic agent (i.e. DBP) in the forming particles. The fixation and binding of microspheres (i.e. agglomerate formation) occurs in

A

B

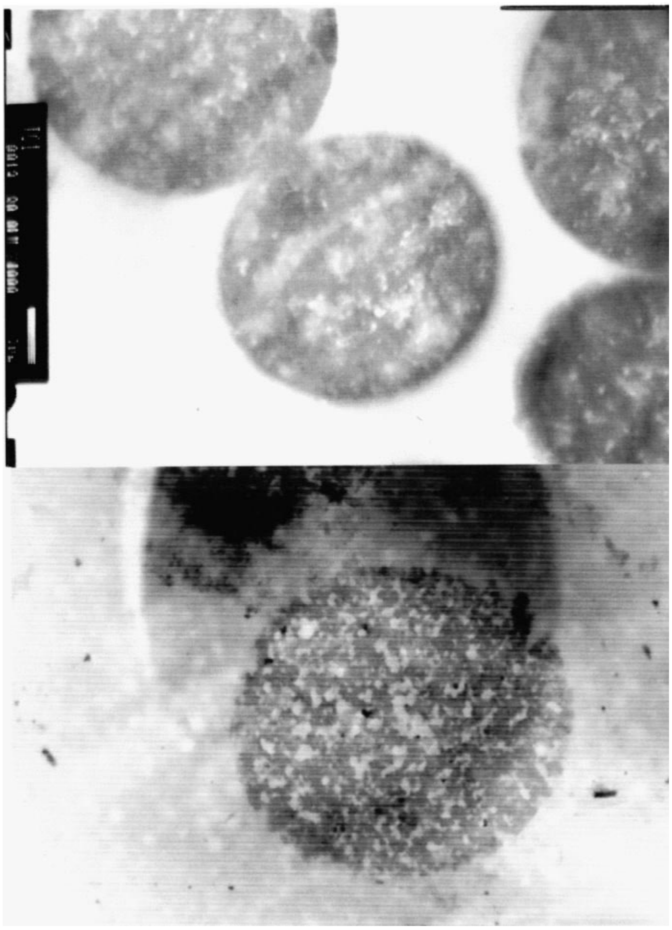

Fig. 4. Typical transmission electron micrographs showing the internal structures of poly(S–VPBA–DVB) particles produced with different (S–DVB)/seed latex ratios, VPBA feed concentration: 5.0 mg/ml, Experiment code and (S–DVB)/seed latex ratio (ml/g): (A) BA2, 9.14 (B) BA5, 5.71, Magnification: ×4000.

the second stage and the voids between the fixed microspheres fill with the porogen solution [24,25]. The voids including porogen solution are the macropores of the uniform particles. After completion of polymerization, the porogen solution is removed from the macropores by washing process. By considering this mechanism, the increase observed in the porosity with decreasing monomer/seed latex ratio (i.e. Fig. 4) should be evaluated as an expected behaviour. Because the amount of porogen solution used for unit mass of final particles was higher in the synthesis performed by utilizing S–DVB/seed latex ratio of 5.71 ml/g.

On the other hand, the lower porosity of VPBA-carrying particles with respect to plain poly(S–DVB) may be explained by the effect of VPBA on the phase separation. The results of studies on the synthesis of functional poly(S–DVB) particles indicated that the presence of relatively polar monomers carrying both hydrophilic and hydrophobic groups makes more difficult the separation of crosslinked microspheres from the porogen phase in the forming uniform particles [31,32]. This effect was particularly observed when the polar monomers like 2-hydroxyethylmethacrylate, methacrylic acid were used together with the S–DVB mixture in the multistage microsuspension polymerization [31,32]. The comonomer, VPBA also includes relatively hydrophilic boronic acid and hydrophobic vinyl–phenyl groups. The agglomeration and binding tendency of crosslinked microspheres increases in the presence of relatively polar monomers which in turn, results in better compatibilization of crosslinked phase with the porogen solution. Hence relatively incomplete separation of crosslinked structure and porogen solution provides a decrease in the porosity of particles [31,32].

In both sets, higher mass–charge densities were observed with the VPBA-carrying particles than those obtained in the absence of VPBA (Table 1). In other words, the mass charge density increased with increasing feed concentration of VPBA under constant polymerization conditions. This behaviour may be evaluated as an indication for the incorporation of VPBA into the final particle structure.

Sample FTIR and FTIR-DRS spectra of poly(S–VPBA–DVB) particles encoded as BA6 were given in Fig. 5. Here, poly(S–DVB) particles produced under identical conditions and without using VPBA were included as a reference material and the same spectra for poly(S–DVB) particles were also given. The characteristic bands located in the FTIR and FTIR-DRS spectra of poly(S–DVB) particles were explained in our previous studies [30–32]. The introduction of VPBA into the particle structure was confirmed by the strong hydroxyl bands appeared at 3500 cm^{-1}, both in the FTIR and FTIR-DRS spectra of poly(S–VPBA–DVB) particles. The relative intensities of the hydroxyl bands (i.e. based on the intensities of aliphatic bands at 2900 cm^{-1}) both in the FTIR and FTIR-DRS spectra of poly(S–VPBA–DVB) particles were close to each other. This finding probably indicated that the bulk and surface concentrations of VPBA were approximately the same.

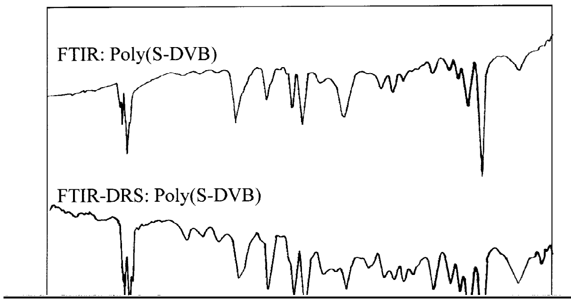

Fig. 5. Typical FTIR and FTIR-DRS spectra of poly(S–DVB) and poly(S–VPBA–DVB) particles produced with the (S–DVB)/seed latex ratio of 5.71 ml/g and VPBA feed concentration of 5.0 mg/ml.

Table 3
Desorption ratios obtained with poly(S–VPBA–DVB) particles loaded with different β-NAD initial concentrations

Desorption ratio (% wt of adsorbed β-NAD) β-NAD initial conc. (μg/ml)	poly(S–DVB)	poly(S–VPBA–DVB)
0.04	94.2	95.6
0.08	94.6	90.1
0.16	93.8	91.8
0.64	90.7	87.4
1.00	92.9	89.6
1.40	91.8	90.2

supports, boronic acid functionality was incorporated into the particles by using a boronic acid-carrying monomer in the polymerization. Hence the stages usually followed (i.e. activation and attachment of boronic acid-carrying ligand) were eliminated. A model molecule (β-NAD) was utilized for testing the reversible adsorption–desorption behaviour of the material against to diol-carrying biomolecules. The usability of particles as an ''affinity-HPLC column material'' in RNA separation is still under investigation.

On the other hand, the size and porosity properties of poly(S–VPBA–DVB) particles are suitable to be a stationary phase material for the affinity-HPLC applications of diol-carrying biomolecules. The uniformity of particles is an advantage for improving the flow-regime and the separation efficiency in the chromatographic columns. While serial column systems in HPLC or successive use of boronate affinity chromatography and HPLC are necessary for the determination of glycated proteins in human serum, the column materials with properties similar to those produced in this study allow the assay of glycated proteins by using a single column in HPLC [42–48].

References

[1] G.L. Shoaf, G.W. Poehlein, Ind. Eng. Chem. Res. 29 (1990) 1701.
[2] S.T. Wang, G.W. Poehlein, J. Appl. Polym. Sci. 50 (1993) 2173.
[3] A. Tuncel, R. Kahraman, E. Piskin, J. Appl. Polym. Sci. 50 (1993) 303.
[4] M.T. Ercan, A. Tuncel, B.E. Caner, E. Piskin, J. Microencapsul. 10 (1993) 67.
[5] A. Tuncel, E. Serpen, Colloid Polym. Sci. 279 (2001) 240.
[6] K. Takahashi, S. Miyamori, H. Uyama, S. Kobayashi, J. Polym. Sci., Part A: Polym. Chem. Ed. 34 (1996) 175.
[7] D. Horak, J. Polym. Sci., Part A: Polym. Chem. Ed. 37 (1999) 3785.
[8] A. Tuncel, R. Kahraman, E. Piskin, J. Appl. Polym. Sci. 51 (1994) 1485.
[9] H. Ayhan, A. Tuncel, N. Bor, E. Piskin, J. Biomater. Sci., Polym. Ed. 7 (1995) 329.
[10] A. Elaissari, Y. Chevalier, F. Ganachaud, T. Delair, C. Pichot, Langmuir 16 (2000) 1261.
[11] E. Castanheira, M.G. Martinho, D. Duracher, M.T. Charreyre, A. Elaissari, C. Pichot, Langmuir 15 (1999) 6712.
[12] T. Delair, F. Meunier, A. Elaissari, M.H. Charles, C. Pichot, Colloids Surf. A: Physiochem. Eng. Aspects 153 (1999) 341.
[13] T. Delair, C. Pichot, B. Madrand, Colloid Polym. Sci. 272 (1994) 72.
[14] M.T. Ercan, A. Tuncel, B.E. Caner, M. Mutlu, E. Piskin, Nucl. Med. Biol. 18 (1990) 218.
[15] M. Okubo, T. Nakagawa, Colloid Polym. Sci. 270 (1992) 853.
[16] S. Margel, E. Nov, I. Fisher, J. Polym. Sci., Part A: Polym. Chem. Ed. 29 (1991) 347.
[17] M. Okubo, Colloid Polym. Sci. 267 (1989) 193.
[18] T. Bahar, A. Tuncel, Polym. Eng. Sci. 39 (1999) 1849.
[19] W.H. Li, H.D.H. Stöver, Macromolecules 33 (2000) 4354.
[20] B. Elmas, S.T. Camli, M. Tuncel, S. Senel, A. Tuncel, J. Biomater. Sci. Polymer Ed. 12 (2001) 283.
[21] T. Ellingsen, O. Aune, J. Ugelstad, S. Hagen, J. Chromatogr. 535 (1990) 147.
[22] Q.C. Wang, F. Svec, J.M.J. Frechet, Science 273 (1996) 205.
[23] J. Ugelstad, Macromol. Chem. 179 (1978) 815.
[24] C.M. Cheng, F.J. Micale, J.W. Vanderhoff, M.S. El-Aasser, J. Polym. Sci., Part A: Polym. Chem. 30 (1992) 235.
[25] C.M. Cheng, J.W. Vanderhoff, M.S. El-Aasser, J. Polym. Sci., Part A: Polym. Chem. 30 (1992) 245.
[26] M. Galia, F. Svec, J.M.J. Frechet, J. Polym. Sci., Part A: Polym. Chem. 32 (1994) 2169.
[27] Q.C. Wang, F. Svec, J.M.J. Frechet, J. Polym. Sci., Part A: Polym. Chem. 32 (1994) 257.
[28] A. Tuncel, M. Tuncel, B. Salih, J. Appl. Polym. Sci. 71 (1999) 2271.
[29] A. Tuncel, J. Appl. Polym. Sci. 71 (1999) 2291.
[30] S.T. Camli, S. Senel, A. Tuncel, J. Biomater. Sci. Polym. Ed. 10 (1999) 875.

[31] T. Camli, M. Tuncel, S. Senel, A. Tuncel, J. Appl. Polym. Sci., (2002) in press.
[32] A. Tuncel, M. Tuncel, B. Ergun, C. Alagöz, T. Bahar, Colloid Surfaces A: Physicochem, Eng. Aspects 197 (2002) 79.
[33] H. Schott, E. Rudloff, P. Schmidt, R. Roychoudhury, H. Kössel, Biochemistry 12 (1973) 932.
[34] R.A. Olsson, J. Chromatogr. 176 (1979) 239.
[35] R.R. Maestas, J.R. Prieto, G.D. Kuehn, J.H. Hageman, J. Chromatogr. 189 (1980) 225.
[36] A. Ozdemir, A. Tuncel, J. Appl. Polym. Sci. 78 (2000) 268.
[37] A. Tuncel, A. Ozdemir, J. Biomater. Sci. Polym. Ed., Ed. 11 (2000) 817.
[38] V. Bouriotis, I.J. Galpin, P.D.G. Dean, J. Chromatogr. 210 (1981) 267.
[39] P.R. Westmark, L.S. Valencia, B.D. Smith, J. Chromatogr. A 664 (1994) 123.
[40] Y.C. Li, E.L. Larsson, H. Jungvid, I.Y. Galaev, B. Mattiasson, Chromatographia 54 (2001) 213.
[41] Y.C. Li, U. Pfüller, E.L. Larsson, H. Jungvid, I.Y. Galaev, B. Mattiasson, J. Chromatogr. A 925 (2001) 115.
[42] K. Yasukawa, F. Abe, N. Shida, Y. Koizumi, T. Uchida, K. Noguchi, K. Shima, J. Chromatogr. 597 (1992) 271.
[43] K. Shima, N. Ito, F. Abe, M. Hirota, M. Yano, Y. Yamamoto, T. Uchida, K. Noguchi, Diabetologia 31 (1988) 627.
[44] R.D. Cristofaro, R. Landolfi, B. Bizzi, M. Castagnola, J. Chomatogr. 426 (1988) 376.
[45] P. Vidal, T. Deckert, B. Hansen, B.S. Welinder, J. Chromatogr. 476 (1989) 467.
[46] J. Jankowski, W. Potthoff, M.V.D. Giet, M. Tepel, W. Zidek, H. Schlüter, Anal. Biochem. 269 (1999) 72.
[47] M. Homma, A.L. Jayewardene, J. Gambertoglio, F. Aweeka, Antimicrob. Agents Chemother. 43 (1999) 2716.
[48] T. Koyama, K. Terauchi, J. Chromatogr. B 679 (1996) 31.
[49] S. Hierten, J.P. Li, J. Chromatogr. 510 (1990) 543.