pH-Sensitive Uniform Gel Beads for DNA Adsorption

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ABSTRACT: Uniform gel beads 3 mm in diameter were obtained by the suspension polymerization of an amine functionalized monomer, *N*-3-(dimethyl amino)propyl-methacrylamide (DMAPM). The polymerization of DMAPM in the form of uniform droplets could be achieved at room temperature in an aqueous dispersion medium by using Ca–alginate gel as the polymerization mold. In this preparation, potassium persulfate/tetramethyl ethylenediamine and sodium alginate/calcium chloride were used as the redox initiator and the stabilizer systems, respectively. The crosslinked DMAPM gel beads exhibited pH-sensitive, reversible swelling–deswelling behavior. The uniform gel beads were also obtained by the copolymerization of DMAPM and acrylamide (AA) in the same polymerization system. Although copolymer gel beads with higher pH sensitivities were obtained with increasing feed concentration of DMAPM, the total monomer conversion decreased. Crosslinked DMAPM and DMAPM–AA copolymer gel beads were utilized as sorbents for DNA adsorption. The gel beads produced with higher DMAPM feed concentration exhibited higher equilibrium DNA adsorption capacity. The DNA equilibrium adsorption capacities up to 50 mg DNA/g dry gel could be achieved with the crosslinked DMAPM gel beads. This value was reasonably higher relative to the previously reported adsorption capacities of known sorbents. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 3154–3161, 2000

Key words: DNA; dimethyl aminopropyl methacrylamide; acrylamide; pH-sensitive gel; cationic gel; anti-DNA antibody

INTRODUCTION

Amine-containing monomers have been widely used in the production of cationic polyelectrolyte gels.[1–5] Siegel et al. studied the production and characterization of gels produced by the copolymerization of *n*-alkyl methacrylate esters and dimethyl aminoethyl methacrylate (DMAEM).[1–4] The swelling process of cationic gels was described by the moving penetrant front mechanism.[2] Chou et al. investigated the dynamic swelling behavior of 2-hydroxyethyl methacrylate-*co*-DMAEM gel structures.[5] Recently, cationic gels which were sensitive to both pH and temperature were prepared by the random copolymerization of *N*-isopropylacrylamide (NIPA) and diethyl aminoethyl methacrylate (DEAEM).[6–7] A mild procedure was also proposed for the production of polycationic gels based on the copolymerization of 2-hydroxyethylmethacrylate and DMAEM.[8] Dimethyl aminopropyl acrylamide (DMAPA) is the another comonomer recently used for the preparation of soluble polymers or gels carrying amino groups. In a study performed by Aoki et al., a terpolymer which was sensitive to both temperature and glucose concentration, was prepared from NIPA, acrylamidophenylboronic

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acid (AcPBA), and DMAPA.[9] Depending on the glucose concentration in the aqueous medium, the prepared terpolymer exhibited different lower critical solution temperature (LCST) values. A glucose responsive gel matrix was also produced by the terpolymerization of NIPA, AcPBA, and DMAPA[10] and used for the investigation of interaction between boronate groups of the gel matrix and diol carrying biomolecules.

In our study, we produced amine funtionalized, pH-sensitive, and uniform gel beads by starting from *N*-[3-(dimethylamino)propyl]methacrylamide (DMAPM) as the main monomer. For this purpose, we employed a polymerization method conducted at room temperature. This method was originally developed by Park and Hoffman for the preparation of thermosensitive and uniform poly(*N*-isopropylacylamide), poly(NIPA) gel beads.[11] A modified version of this method was used for the preparation of enzyme entrapped poly(NIPA) gel spheres by us.[12–13] Finally the method was adopted for the production of pH-sensitive and amine-functionalized gel spheres in this study.

On the other hand, different materials have been proposed as solid supports for the adsorption or immobilization of DNA onto polymeric surfaces.[14–25] It was shown that the polyion complexation between DNA and amine groups of the carrier matrix provided more quantitative and stable immobilization of DNA relative to the other interactions.[23] For this reason, amine-carrying polymeric structures have been employed as solid supports in DNA adsorption or immobilization studies.[21–24] The amine functionality could be incorporated with diamine-carrying ligands by the chemical activation of reactive groups on the surface of support materials (hydroxyl, carboxyl, etc.) with the proper agents (i.e., CNBr, tosyl chloride, or water-soluble carbodiimide).[21–22] One of the recent methods used for the generation of amine groups was graft polymerization performed after ozonization of the support material.[23–24] The common feature of these methods is that the base of the support matrix is usually an inert material like polystyrene, agarose, or poly(ethylene terephthalate) PET.[20–25] Then, a limited number of amine groups interacting with the DNA molecules can be generated depending on the yield of activation process and the number density of activated functional groups on the support surface. Instead of this approach, we planned to use a matrix completely made of an amine-funtionalized material and then selected DMAPM-based gel spheres as a sorbent for DNA adsorption. This approach provided DNA adsorption capacities up to 50 mg/g dry gel, which was a much higher value relative the those of known sorbents.

In this study, we aimed at the synthesis of sorbents having high DNA adsorption capacity. In the therapy of some autoimmune diseases, such as systemic lupus erythematosus (SLE), the removal of anti-DNA antibodies from the plasma of patient can be achieved by utilizing DNA immobilized polymeric sorbents.[26–29] The decrease in the plasma level of anti-DNA antibody may lead to a clinical improvement.[25] The presence of a higher amount of DNA immobilized on the surface of sorbent material usually provides higher anti-DNA antibody adsorption capacity.[23,25] Then higher anti-DNA antibody removal rate is possible with a sorbent with higher DNA content. Here, owing to their reasonably high number density of related functional groups (i.e., dimethylamino) which could interact with DNA molecule, poly(DMAPM) gel beads were examined as a sorbent to achieve reasonably high DNA immobilization capacities.

EXPERIMENTAL

Materials

The monomers, DMAPM (Aldrich Chemical Co., Milwaukee, WI) and acrylamide (AA; BDH Chemicals Ltd., Poole, England) were utilized without further purification. *N*,*N*-Methylenebisacrylamide (MBA; BDH Chemicals Ltd.) was used as the crosslinker. In the preparation of gel beads, potassium persulfate (KPS; BDH Chemicals Ltd.) and tetramethyl ethylene diamine (TEMED; Sigma Chemical Co., St. Louis, MO) were selected as the initiator and the accelerator, respectively. Sodium alginate (medium viscosity; Sigma) and calcium chloride (BDH Chemicals Ltd.) were used for the formation of alginate mold around the spherical gel beads. Deoxyribonucleic acid (DNA; Salmon Testes, Cat. No. D1626) was supplied from Sigma Chemical Co. Distilled deionized water was used in all experiments.

Preparation of Gel Beads

A typical procedure for the preparation of pH-sensitive DMAPM gel beads is exemplified below. The continuous medium was prepared by dissolv-

ing KPS (400 mg) and $CaCl_2$ (1200 mg) in distilled deionized water (40 mL). The medium was purged with bubbling nitrogen for 1 h before the injection of the dispersed phase, including monomer, crosslinker, and stabilizer. For the preparation of the dispersed phase, DMAPM (1 mL), MBA (10 mg), and Na alginate (30 mg) were dissolved in distilled deionized water (2.5 mL). Then the accelerator (TEMED, 0.25 mL) was added into the resulting homogeneous mixture. The dispersed phase was dropped by a system including a syringe and a dosage pump into the continuous medium which was kept at 20°C and stirred magnetically with 250 rpm. The polymerization was conducted at 20°C with the same stirring rate for 4 h. Uniform, pH-sensitive gel beads 3 mm in size were obtained. The gel beads were filtered and washed extensively with distilled water and stored in the refrigerator at +4°C until use. For the preparation of DMAPM–AA copolymer gel beads, the same recipe was also applied except the DMAPM/AA weight ratio was changed between 0/100 and 100/0 in the dispersed phase by fixing the total amount of DMAPM and AA to 1 g.

Characterization of Gel Beads

The surface morphology and the internal structure of the gel beads were observed by a scanning electron microscope (Jeol, JEM 1200EX, Japan). For this purpose, the gel beads were swollen to equilibrium in citrate buffer (pH 3.0) and subsequently freeze-dried at −20°C. The freeze-dried beads were cut to obtain the cross-sectional view of the internal structure. The samples, coated with a thin layer of gold (about 100 A) *in vacuo,* were examined by a scanning electron microscope.

The total monomer conversion into the uniform gel bead form (i.e., bead yield) was found by the determination of the dry weight of gel beads. For this purpose, the gel beads obtained from one batch were washed and then dried in a vacuum oven at 60°C for 48 h. The bead yield was calculated by taking the ratio of the dry weight of gel beads to the initial monomer weight (including MBA).

The variation of the equilibrium swelling ratio of poly(DMAPM) or poly(DMAPM-*co*-AA) gel beads with the medium pH was studied in the buffer media at room temperature (i.e., 22°C). For this purpose, HCl, citrate, phosphate, and carbonate buffer solutions were used to obtain pH values between 1.2 and 10.0. A typical procedure is given as follows. The washed gel beads obtained from one batch (approximately 3 mm in diameter in the production conditions) were equilibrated in 0.1*N* HCl solution (100 mL) for 24 h at room temperature and the weight of the swollen gel was measured after removing excess water by a filter paper. The gels were transferred to a new buffer medium (100 mL) having a higher pH value (i.e., 2.0) after they were extensively washed. At the end of an equilibrium period of 8 h, the equilibrium weight of the gel was again recorded. The equilibrium swelling ratio measurements were repeated with this procedure, increasing the medium pH up to 10.0. The equilibrium swelling ratio of gel beads at any pH was calculated by eq. (1), where ϕ is the equilibrium swelling ratio of gel beads at a particular pH, M_s (g) is the swollen weight of gel beads at equilibrium, and M_d (g) is the dry weight of gel beads, such that

$$\phi = (M_s - M_d)/M_d \quad (1)$$

The dynamic response of gel beads was determined in a separate group of experiments. To follow the dynamic shrinking behavior, a step input of the medium pH with a magnitude of 4 units was applied by transferring the gel beads equilibrated in citrate buffer (pH 3.0, ionic strength: 0.1) into a phosphate buffer solution (pH 7.0, ionic strength: 0.1). Then the weight of gel beads was determined at designated times. The dynamic swelling behavior was monitored by applying a step input having the same magnitude of the medium pH, but in the reverse direction (−4 units). In this experiment, the gel beads equilibrated at pH 7.0 were transferred into citrate buffer solution having a pH of 3.0. Ionic strength of these solutions were also fixed at 0.1.

DNA Adsorption Experiments

The adsorption isotherm of DNA was obtained by using pure poly(DMAPM) gel beads as the sorbent. For this purpose, DNA initial concentration in the adsorption medium was changed between 0.25 and 2.0 mg/mL. A typical procedure followed for DNA adsorption may be given as follows. To prepare the adsorption medium, a certain amount of DNA was dissolved in phosphate buffer solution (40 mL, pH 7.4, ionic strength: 0.1) by stirring the medium with 200 rpm for 24 h at +4°C. Then, poly(DMAPM) gel beads (approximate dry weight: 0.30 g) equilibrated in a phosphate buffer solution were transferred into the adsorption me-

dium including DNA with a certain initial concentration. The adsorption process was conducted at +4°C, with 200 rpm stirring rate for 8 h (i.e., a safe equilibrium adsorption period determined by the preliminary experiments). To determine the equilibrium DNA adsorption capacity of gel beads, initial and final DNA concentrations in the adsorption medium were measured according to the method developed by Spirin.[30] A 0.1 mL DNA sample solution was put into the aqueous perchloric acid solution (0.25*N*, 5 mL), and the resulting mixture was heated to 90°C and kept at this temperature for 20 min to break the DNA into small nucleotides. After cooling down to room temperature, the absorbances of the solution were measured at both 270 and 290 nm in a UV–Vis spectrophotometer (Hitachi, Japan) against 0.25*N* $HClO_4$ solution as the control. By using the calibration curve between known DNA concentration and $\Delta A = (A_{270} - A_{290})$ values, the concentration of DNA in the assayed sample was determined. The equilibrium DNA adsorption capacity (Q, mg DNA/g dry gel) was calculated based on the expression:

$$Q = (C_o - C_f)V/M_o \quad (2)$$

Here, C_0 and C_f (mg DNA/mL) are the concentrations of DNA in the adsorption medium before and after the adsorption process, respectively. V (mL) is the volume of the adsorption solution and M_0 (g) is the dry weight of gel beads determined after completion of adsorption experiment, by drying the gel beads at 60°C for 48 h in a vacuum oven. Equilibrium DNA adsorption experiments were also performed by using gel beads produced with different DMAPM/AA feed ratios between 100/0 and 0/100. These experiments were also done under identical conditions.

RESULTS AND DISCUSSION

Characterization of pH-Sensitive Gel Beads

Poly(DMAPM) and poly(DMAPM-*co*-AA) gel beads 3 mm in size were obtained in monodisperse form by using Ca–alginate gel as the polymerization mold. Photographs of uniform poly(DMAPM) gel beads in acidic and neutral media are given in Figure 1. As seen here, the gel beads were responsive to pH change. While they were in the swollen form in the acidic medium (pH 3.0) because of the ionization of dimethylamino groups, they significantly collapsed in the neutral medium (pH 7.4).

The surface morphology and the internal structure of poly(DMAPM) gel beads are exemplified by the electron micrographs given in Figure 2. As seen in the electron micrograph taken with ×800 magnification, the gel beads comprised a highly macroporous interior and a shell placed around this matrix [Fig. 2(A)]. Note that these micrographs were taken with the freeze-dried forms of gel beads equilibrated at a pH of 3.0. Therefore the electron micrograph in Figure 2(A) shows the macroporous structure of swollen gel beads. The internal structure of gel beads was also very similar to that of crosslinked poly(NIPA) gel prepared by a similar polymerization method.[12] However, the shell side was not as macroporous as that of the internal part [Fig. 2(B)]. Because Na–alginate and $CaCl_2$ were used for the formation of alginate mold around the gel beads, the shell may be considered as an interpenetrating network comprising Ca–alginate and poly(DMAPM) chains.

The variation of equilibrium swelling ratio with medium pH is given in Figure 3 for the copolymer gel beads prepared with different DMAPM/AA feed ratios. As seen here, the equilibrium swelling ratio difference between the fully swollen and fully collapsed states decreased with the decreasing feed concentration of pH-responsive component (i.e., DMAPM). As expected, the best pH sensitivity was observed with the poly (DMAPM) gel beads. The medium pH was not as effective on the equilibrium swelling ratio of gel beads prepared with the AA feed concentration of 48.5% or higher. To observe the dynamic shrinking behavior of gel beads, a step input was applied by changing the medium pH from 3 to 7. The variation of swelling ratio with the time under the applied step input is given in Figure 4. The dynamic swelling behavior was monitored by applying the step input having the same magnitude on the medium pH, but in the reverse direction. The results indicated that swelling and contraction rates (i.e., the variation of dimensionless equilibrium water content with the time) were approximately equal and both processes were completed within about 4 h.

The variation of monomer conversion into the bead form (i.e., the bead yield) with the DMAPM/AA feed ratio is given in Figure 5. As seen here, the bead yield significantly increased with increasing feed concentration of AA. Although a bead yield of 85% was obtained when

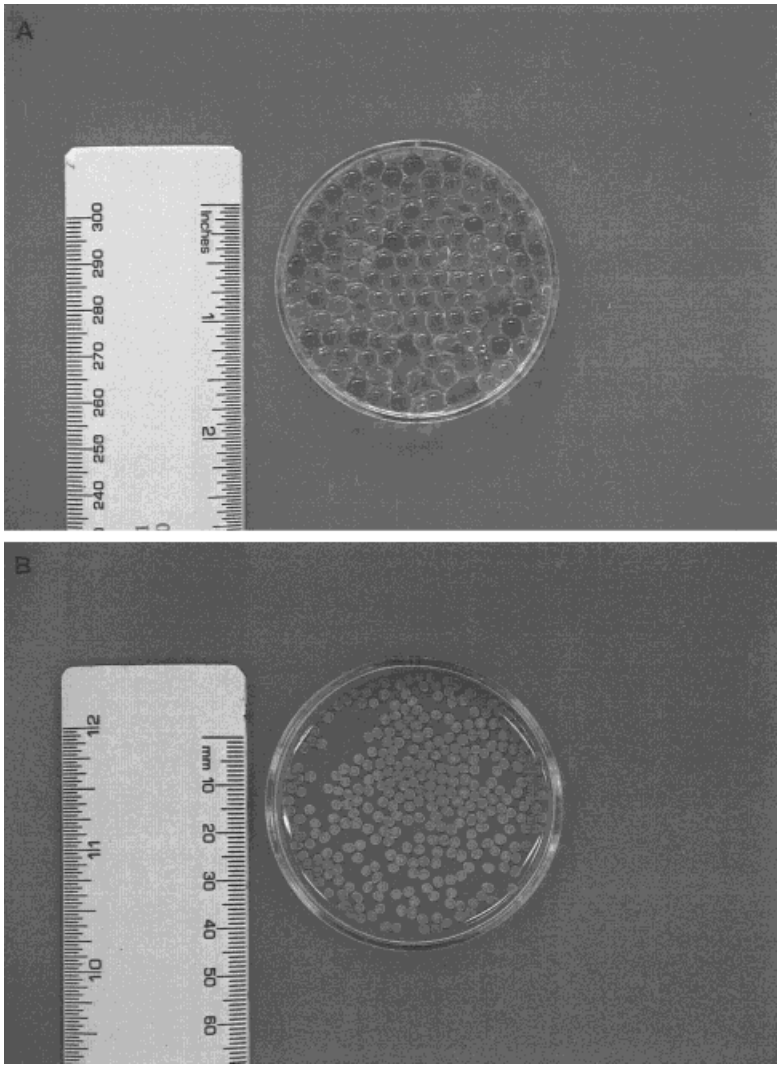

Figure 1 Photographs of uniform poly(DMAPM) gel beads in (A) acidic and (B) neutral media.

only AA was used as the monomer, the bead yield was 42% in the presence of pure DMAPM.

DNA Adsorption Experiments

In the first set of these experiments, DNA initial concentration was changed between 0.25 and 2.0 mg/mL by using poly(DMAPM) gel beads as the sorbent. The temperature and pH were fixed at +4°C and 7.4, respectively. The variation of equilibrium DNA adsorption capacity with the initial DNA concentration is given in Figure 6. As seen here, the equilibrium adsorption capacity markedly increased up to the initial DNA concentration of 1 mg/mL. The plateau value of equilibrium adsorption capacity was approximately 50 mg/g dry gel. This value was reasonably higher relative to the results reported in the literature. Kato and Ikada studied DNA adsorption onto PET microfibers with a high specific surface area of 0.83 m^2/g.[23] After ozone oxidation, the microfibers were exposed to graft polymerization of monomers including AA, methacryoyloxyethyl phosphate, *N*,*N*-dimethyl aminoethyl methacrylate, *N*-vinyl formamide, and glycidyl methacrylate. The highest DNA equilibrium adsorption capacity was obtained as approximately 5 mg DNA/g with the poly(methacroyloxyethyl phosphate)-grafted PET microfibers (i.e., the value calculated from the reported value of 0.6 μg DNA/cm^2).[23] On the other hand, the maximum equilibrium DNA adsorption

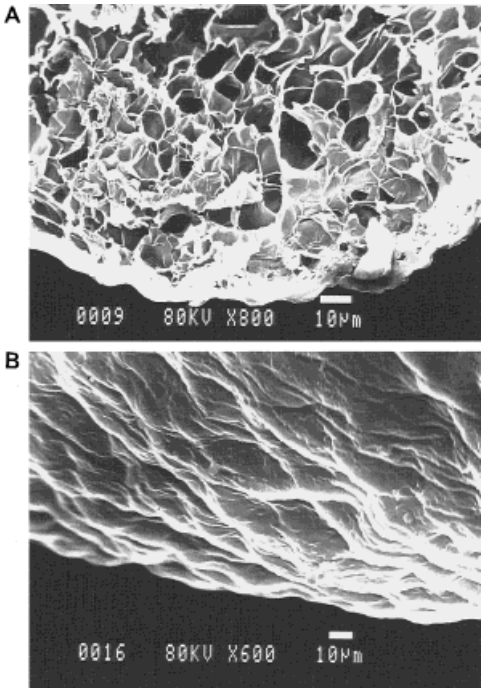

Figure 2 The electron micrographs showing (A) the internal structure (magnification, ×800) and (B) the surface morphology (magnification, ×600) of poly(DMAPM) gel beads.

capacity was obtained with the activated poly(2-hydroxyethylmethacrylate) microbeads as 2.75 mg DNA/g microbeads[25]. The complex formation

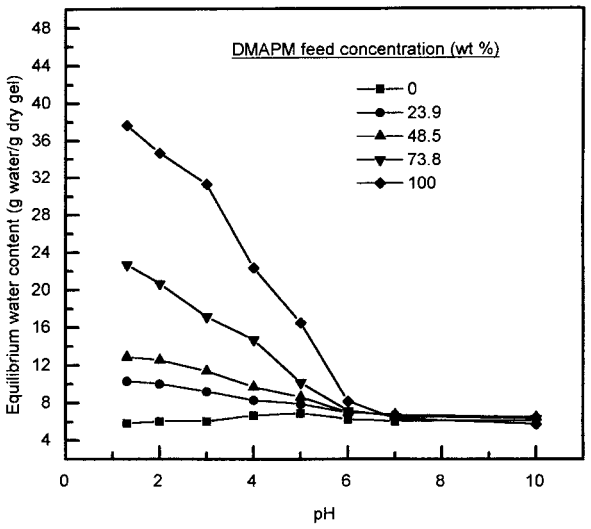

Figure 3 The variation of equilibrium swelling ratio with the medium pH for the poly(DMAPM-*co*-AA) gel beads produced with different DMAPM weight fractions in the feed mixture.

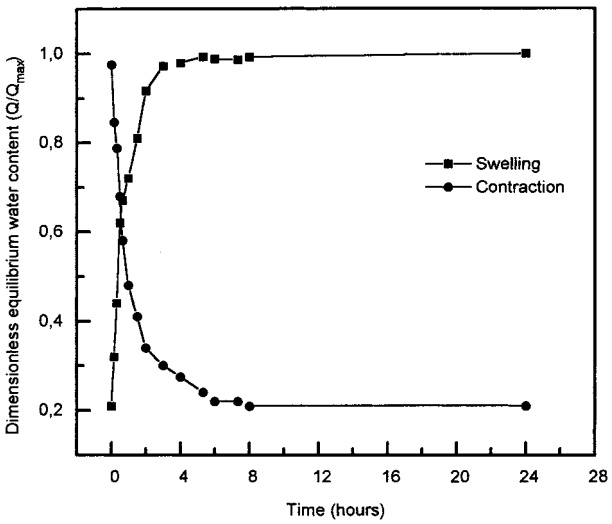

Figure 4 The dynamic swelling and shrinking behaviors of poly(DMAPM) gel beads.

ability between the dimethylamino groups of sorbent materials and phosphorus-containing groups of DNA molecules are mostly used for the binding of DNA molecules onto the solid support materials.[21,23] The high DNA adsorption capacity observed in our study may be explained by the higher dimethylamino content of the gel beads. In some of the studies performed elsewhere, the sorbents for DNA adsorption were prepared by introducing the dimethylamino functionality into selected base materials by chemical activation or by graft polymerization.[21–24] Because the gel used as

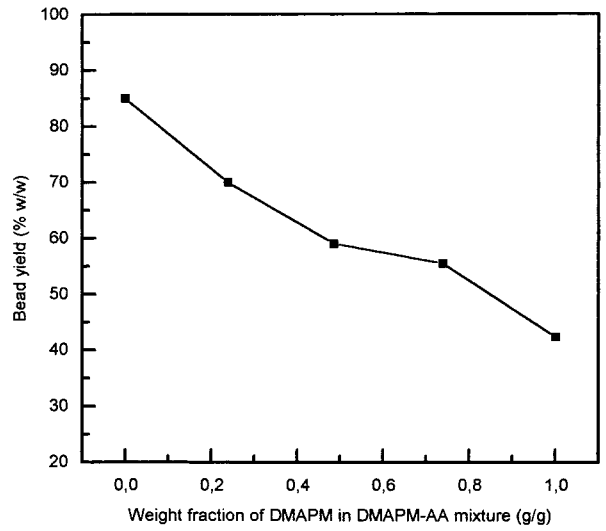

Figure 5 The variation of monomer conversion into the bead form with the DMAPM/AA feed ratio.

a sorbent in our study was directly produced by the polymerization of a dimethylamino-carrying monomer (i.e., DMAPM), the dimethylamino content of the sorbent material was expected to be higher relative to those obtained by the activation or grafting procedures. Therefore, such a higher adsorption capacity (i.e., 50 mg DNA/g dry gel) could be achieved with the proposed sorbent.

In the second set, the gel beads produced with different DMAPM/AA feed ratios were used as sorbents and their equilibrium DNA adsorption capacities were determined at a pH of 7.4 at +4°C with the initial DNA concentration of 1.5 mg/mL. The variation of equilibrium DNA adsorption capacity with the weight fraction of DMAPM in DMAPM–AA feed mixture is given in Figure 7. As seen here, the highest equilibrium DNA adsorption capacity was obtained with the poly (DMAPM) gel beads. On the other hand, the equilibrium DNA adsorption capacity of poly(AA) gel beads was very low. The introduction of DMAPM into the gel structure resulted in an appreciable increase in the equilibrium DNA adsorption capacity. However, this increase was not directly proportional to the weight fraction of DMAPM in the feed mixture. In the selected production method, although AA was converted into the bead form with a reasonably high yield (i.e., 85%), the conversion of DMAPM into bead form was reasonably low (i.e., 42%) (Fig. 5). In other words, the conversion of AA into the bead form was easier relative to DMAPM in the selected polymerization method. Owing to this behavior, the final DMAPM content of DMAPM–AA copolymer gel beads was probably lower than that used in the feed mixture. This may be the reason for the tendency observed with the DMAPM–AA copolymer gel beads in Figure 7.

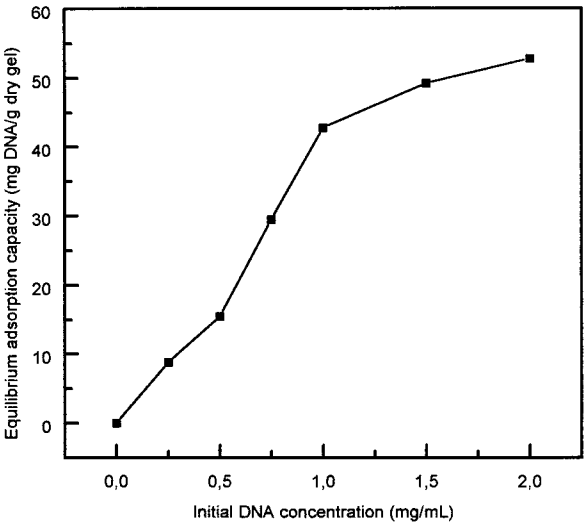

Figure 6 The variation of equilibrium DNA adsorption capacity of poly(DMAPM) gel beads with the initial DNA concentration.

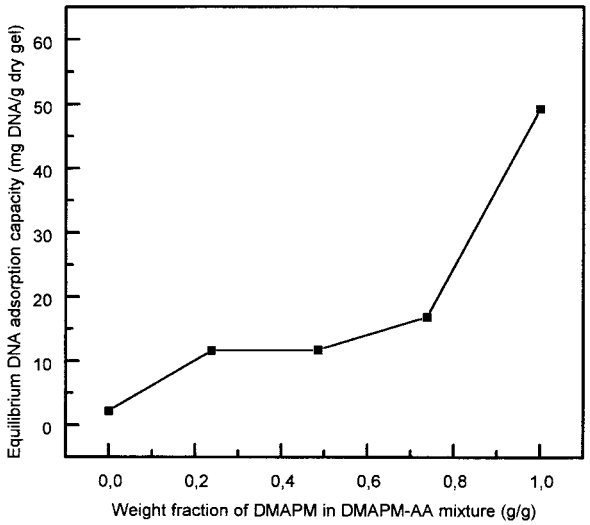

Figure 7 The variation of equilibrium DNA adsorption capacity with the weight fraction of DMAPM in DMAPM–AA feed mixture.

CONCLUSION

Uniform gel beads exhibiting pH-sensitive reversible swelling behavior were obtained by suspension polymerization of DMAPM. By considering the complex formation between phosphorus-containing groups of DNA and dimethylamino groups, gel beads were tried as a sorbent for DNA adsorption. Reasonably higher equilibrium DNA adsorption capacities (i.e., up to 50 mg/g dry gel) could be achieved with the proposed sorbent. The removal of anti-DNA antibodies by DNA immobilized sorbents is a common approach utilized in the therapy of some autoimmune diseases.[26–29] The equilibrium anti-DNA antibody adsorption capacity was shown to be proportional to the amount of DNA immobilized on the surface of tried sorbents.[23,25] The sorbents carrying higher amounts of DNA may be considered good candidates for achieving higher removal rates for anti-DNA antibodies.

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