Boronic Acid–Functionalized HEMA-Based Gels for Nucleotide Adsorption

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ABSTRACT: A gel matrix that could be used as a sorbent for the specific adsorption of nucleotides was prepared by the radical copolymerization of 2-hydroxyethylmethacrylate (HEMA) with a relatively new boronic acid–functionalized monomer (4-vinylphenylboronic acid, VPBA). The synthesis of a gel could be achieved at a reasonably low temperature (+4°C) by using potassium persulfate and tetramethylethylenediamine as the redox system and methylenebisacrylamide (MBA) as the crosslinker. To increase the diol binding affinity of boronic acid–carrying gels, two different amine-containing monomers [*N*-3-(dimethylamino)propylmethacrylamide, DMAPM and 2-(dimethylamino)ethylmethacrylate, DMAEM) were also included in the gel-formation recipe. Then HEMA–VPBA–DMAPM and HEMA–VPBA–DMAEM terpolymer gels were obtained. The boronic acid–functionalized gel matrices with different swelling properties were produced by changing the feed concentrations of VPBA and of the amine-containing monomers (DMAPM and DMAEM). To test the usability of produced gels as a sorbent in the nucleotide adsorption, β-nicotinamide adenine dinucleotide (β-NAD) was selected as a model compound. The results of adsorption experiments indicated that the β -NAD adsorption capacity of HEMA-based gels increased with increasing VPBA feed concentration. Equilibrium adsorption capacities up to 33 mg β-NAD/g dry gel could be achieved with the DMAPM-containing boronic acid–functionalized HEMA-based gels. An increase in the feed concentration of DMAPM resulted in an increase in the β-NAD adsorption capacity of gels, while a decrease was observed with an increasing feed concentration of the other amine-containing monomer (DMAEM). © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 78: 268–277, 2000

Key words: hydroxyethylmethacrylate; aminophenylboronic acid; vinylphenylboronic acid; (dimethylamino)propylmethacrylamide; nucleotide; glycoprotein

INTRODUCTION

Boronic acid–functionalized gels have attracted significant attention in the affinity chromatography of various biological agents. Affinity chromatography applications have been developed based on the complex formation between boronic acid and the cis–glycol groups of the biological agent. Schott proposed a synthesis method for tetramethylene dimethacrylate–based boric acid gel and used the produced gel matrix in the separation of ribonucleoside–deoxyribonucleoside mixtures.[1] Also investigated were the properties of a dihydroxyboryl-substituted methacrylic acid polymer and its application to the column chromatographic separation of nucleic acid components.[2] Bio-gel P-2–based boronic acid gel was proposed as a stationary phase for the analysis of ribonucleosides by reversed-phase high-performance liquid chromatography (HPLC).[3] Phenyl(dihydroxyboryl) polyacrylamide beads were also synthesized starting from Bio-Gel P2 hydrazide

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beads and utilized in the chromatography of ribonucleosides.[4]

Similar gels were also tried as support materials in the affinity chromatography of enzymes. Boronate gels carrying different cofactors (flavine adenine dinucleotide or the oxidized and reduced forms of nicotinamide adenine dinucleotide) were utilized as a stationary phase in the purification of uridine 5′-diphosphate glucose pyrophosphorylase.[5] These gels were prepared by starting from commercial aminoethyl polyacrylamide beads (i.e., P-2 and P-150). The purification of glucose-6-phosphate dehydrogenase could be achieved by an agarose-based boronic acid gel carrying similar nucleotides.[6] Boronic acid–functionalized gels were also tried as a stationary phase in the quantitative detection of glycated hemoglobin and glycosylated albumin.[7–9]

In most of these studies, a two-step synthesis procedure was utilized for the preparation of boronic acid–functionalized gel matrices. In the first step, a base matrix synthesized or commercially supplied was reacted with a proper activation agent (cyanogen bromide, a water-soluble carbodiimide, or 1,4-butanediol diglycidyl ether), depending on the type of available functional groups. Then an agent carrying boronic acid residues (mostly *m*-aminophenylboronic acid, APBA) was covalently coupled onto the activated gel structure via the amine groups of this ligand.[1–9]

As an alternative, boronic acid–carrying gels can be prepared by applying single-stage synthesis procedures.[10–15] In these procedures the acrylate-type hydrophilic monomers (acrylamide or isopropylacrylamide) are directly copolymerized with a boronic acid–functionalized acrylate-based monomer, acrylamidophenyl boronic acid, synthesized by starting from APBA and acrylic acid.[10–15]

In our previous studies we produced 2-hydroxyethylmethacrylate-based (HEMA-based) gels with different functionalities for biotechnological applications.[16–19] In the current study we aimed for the direct synthesis of a boronic acid–carrying gel structure. By considering the potential use of the developed matrix in the biotechnological and medical applications, we planned to use a nontoxic and reasonably biocompatible base and selected 2-hydroxyethylmethacrylate (HEMA) as the main component of the gel matrix. Then we copolymerized HEMA with a reasonably new and commercially available monomer carrying boronic acid residues (4-vinylphenylboronic acid, VPBA). The ternary copolymers of HEMA, VPBA, and amine-containing monomers [*N*-3-(dimethylamino)propylmethacrylamide, DMAPM, and 2-(dimethylamino)ethylmethacrylate, DMAEM] were also prepared in order to obtain gel structures with a higher binding affinity for diol-containing biomolecules. A nucleotide, β-nicotinamide adenine dinucleotide (β-NAD), was selected as the model diol-containing biomolecule, and the adsorption of β-NAD onto the produced gel structures was investigated.

EXPERIMENTAL

Materials

The monomers 2-hydroxyethylmethacrylate (HEMA), *N*-3-(dimethylamino)propylmethacrylamide (DMAPM), 2-(dimethylamino)ethylmethacrylate (DMAEM), and 4-vinylphenylboronic acid (VPBA) were supplied from Aldrich Chem. Co., Milwaukee, WI, and were used without further purification. *N,N*-Methylenebisacrylamide (BDH Chemicals Ltd., Poole, UK) was selected as the crosslinker in the gel preparation. The copolymerization was initiated with a potassium persulfate (KPS, BDH Chemicals Ltd.) and tetramethylethylenediamine (TEMED, Sigma Chemical Co., St. Louis, MO) redox system. β-Nicotinamide adenine dinucleotide (β-NAD, Sigma Chemical Co.) was used in the adsorption experiments. The buffer solutions for the adsorption experiments were prepared by using *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid (HEPES, Sigma Chem. Co.). Distilled deionized water was used in all experiments.

Preparation of Gel Matrix

A typical gel preparation may be described as follows: HEMA (1.0 mL), VPBA (0.05 g), DMAPM (0.4 mL), and MBA (0.005 g) were dissolved in 1.5 mL of distilled–deionized water in a cylindrical Pyrex tube 8 mm in internal diameter. After adding aqueous TEMED solution (0.25 mL, 10% by volume), the resulting medium was put into a thermostated water bath at 4°C. After establishing thermal equilibrium, KPS solution (0.25 mL, 50 mg/mL) was added into the pregel solution, and the tube was purged with bubbling nitrogen for 2 min and sealed. The gel formation was conducted at 4°C for 24 h under a nitrogen atmosphere. The gels were removed from the tubes by applying pressure and cut into discs 4 mm in height and 8 mm in diameter under production conditions. The gel discs were washed extensively

with distilled water and used in the equilibrium swelling and β-NAD adsorption experiments. To provide gel matrices with different swelling properties, and β-NAD adsorption characteristics, the VPBA concentration and the type and concentration of the amine-containing comonomer in the gelation medium were changed. The monomer conversions, determined by measuring the dry weight of the gel samples, showed that nearly quantitative monomer conversion (higher than 95%) could be achieved in all preparations.

Characterization of Gels

The variation of equilibrium swelling ratio with pH of prepared gel matrices in aqueous buffer media at room temperature (22°C) was studied. For this purpose, buffer solutions with pH values between 2.0 and 8.6 were utilized. The total ionic strength was fixed at 0.1 in all solutions. A typical procedure followed in the equilibrium swelling experiments is as follows: The washed gel samples were cut into discs 4 mm in height and 8 mm in diameter under production conditions and were placed into 100 mL of a buffer solution having a certain pH value. Then the gel samples were swollen/collapsed up to the equilibrium for 24 h at room temperature. The weight of swollen gel (W_s) was measured with an electronic balance with a $\pm$0.01 g accuracy after removing the excess surface water by a filter paper. The swollen gel samples were dried *in vacuo* at 60°C for 2 days, and the dry weight (W_d) was determined. The equilibrium swelling ratio was calculated based on the following expression:

$$Q = (W_s - W_d)/W_d \quad (1)$$

To investigate the response of produced gels against diol-containing molecules, glucose was also selected as a model molecule, and the equilibrium swelling experiments were repeated within the buffer solutions with the same pH values and containing 1% and 10% w/v of glucose. In these experiments gels prepared with different VPBA and DMAPM contents were examined.

β-NAD Adsorption Experiments

In the β-NAD adsorption experiments, performed in batch fashion, gels produced with different concentrations of VPBA and amine-containing monomers (e.g., DMAPM and DMAEM) were used. In a typical adsorption experiment, a single gel tablet (4 mm in height and 8 mm in diameter in production conditions, with a dry weight of approximately 0.1 g) were put into the HEPES buffer (40 mL, pH 8.5) including 0.05*M* $MgCl_2$ at 4°C. The equilibrium adsorption experiments were conducted at 4°C for 24 h with a 200-rpm stirring rate. For the derivation of β-NAD adsorption isotherms, the initial β-NAD concentration in the adsorption medium was varied between 0.01–0.48 mg/mL. The equilibrium adsorption capacity of the boronic acid–functionalized gels was determined by a conventional spectrophotometric method by measuring initial and final absorbance of the adsorption medium in a UV–vis spectrophotometer (Hitachi, Japan) at a wavelength of 260 nm.[6] Unless stated otherwise, identical conditions were used in the other β-NAD adsorption experiments done to test the effects of gel properties on the equilibrium β-NAD adsorption capacity.

β-NAD Desorption Experiments

For the removal of adsorbed β-NAD from the gel matrix, a desorption medium reported on in the literature was used.[5] β-NAD-adsorbed gel samples prepared with different β-NAD initial concentrations in the adsorption medium were transferred into 40 mL of desorption medium with a pH of 10.0 at 4°C (0.1*M* borate buffer solution, including 0.1*M* NaCl). Then the medium was stirred for 8 h at 4°C with 200 rpm. The final absorbance of this medium was measured at 260 nm. By using the calibration curve prepared in the borate buffer medium (β-NAD concentration versus absorbance), the desorption ratio was calculated as the ratio of the desorbed amount of β-NAD to the adsorbed amount of β-NAD on the gel.

RESULTS AND DISCUSSION

Characterization of Gel Matrices

Equilibrium swelling experiments were performed in aqueous buffer solutions with pH values between 2.0 and 8.6. Swelling experiments were also repeated by using the same buffer solutions, which had glucose concentrations of 1.0 and 10 % w/v. In these experiments the upper pH limit was selected as the pKa value of phenylboronic acid (pH 8.6).[12,14] For poly(HEMA) and boronic acid–functionalized poly(HEMA) gels, the variations of equilibrium swelling ratio in relation to medium pH is described in Figure 1. Here

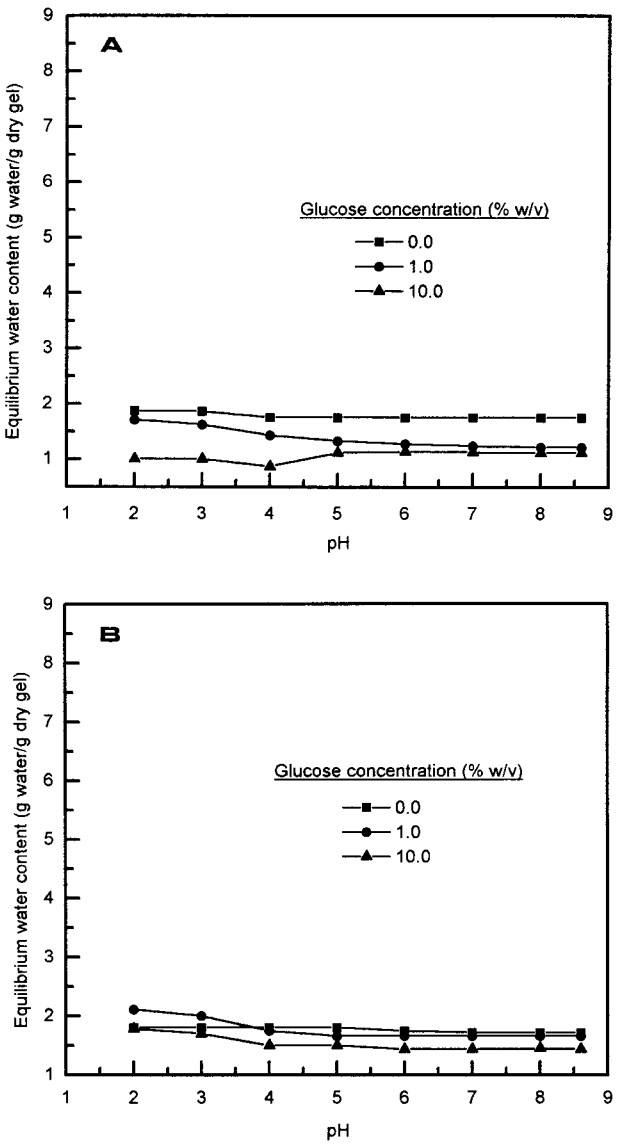

Figure 1 The variation of equilibrium swelling ratio with medium pH for (a) poly(HEMA) and (b) boronic acid–functionalized poly(HEMA) gels.

the equilibrium swelling behavior of poly(HEMA) gel is included as a reference. As expected, no significant change was observed in the equilibrium swelling capacity of poly(HEMA) gel from medium pH. The swelling behavior of poly(HEMA-*co*-VPBA) gel prepared by the VPBA feed ratio of 2.6 mol % is given in Figure 1(b). As seen there, the swelling behavior of this gel was very similar to that of poly(HEMA) in the selected pH range. The absence of a significant change in the equilibrium swelling ratio of the copolymer gel at the pKa value of phenylboronic acid (pH 8.6) may be attributed to the swelling behavior of VPBA–HEMA gel being dominantly controlled by the HEMA segments in the copolymer structure.

In the previously published studies, significant changes in the lower critical solution temperature (LCST) of thermoresponsive forms of boronic acid–functionalized linear polymers (isopropylacrylamide-acrylamidophenylboronic acid copolymers) were reported as a specific response against the concentration of a model diol compound (glucose).[12,14] By starting from this point and by taking into account the possible effect of glucose concentration on the swelling behavior of HEMA–VPBA gel, the equilibrium swelling experiments were repeated in the buffer media containing glucose. However, the equilibrium swelling ratio at a certain pH slightly decreased with the increasing glucose content of the swelling medium for both gels. This result indicated that boronic acid–functionalized HEMA gel does not exhibit any significant change in the equilibrium swelling capacity as a specific response against a diol-carrying compound (e.g., glucose).

In some recent studies it was reported that the introduction of an amino group into phenylborate polymers was quite effective for increasing the complexation ability and the glucose responsivity at physiological pH.[14,15] Then boronic acid–functionalized HEMA-based gels were also prepared in the form of terpolymers by the use of two amine-carrying comonomers, which were selected as *N*-3-(dimethylamino)propylmethacrylamide (DMAPM) and 2-(dimethylamino)ethylmethacrylate (DMAEM). The swelling behaviors of HEMA–VPBA–DMAPM (HBAp) and HEMA–VPBA–DMAEM (HBAe) terpolymer gels are described, respectively, in Figure 2(a,b). In the preparation of these gels, VPBA and amine-carrying comonomer (DMAPM or DMAEM) feed concentrations were fixed to 2.6 and 20.4 mol %, respectively. As seen here, higher equilibrium swelling values were obtained in the acidic pH region because of the ionization of basic dimethylamino groups in the structure of both gels. However, the swelling response against glucose concentration was reasonably similar to those of the previous gel structures.

β-NAD Adsorption Isotherm

As emphasized previously, the gels carrying reactive groups (usually amine or hydroxyl) were activated with the proper agents, and the boronic acid functionality in the resulting matrix was obtained by the chemical interaction of the activated gel structure with a boronic-acid-carrying

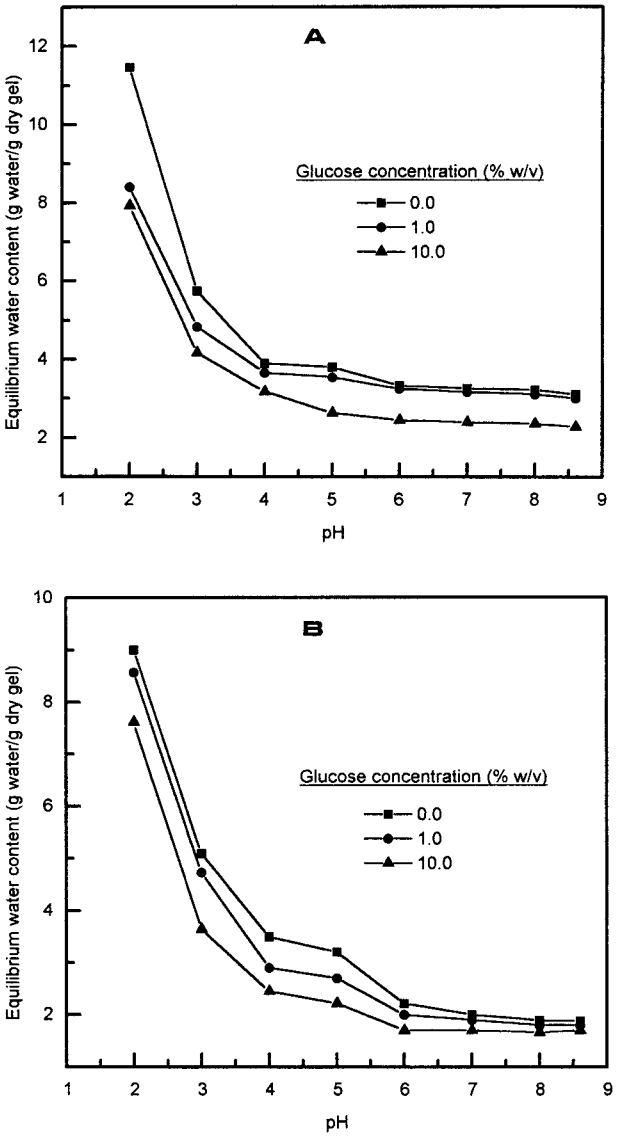

Figure 2 The variation of equilibrium swelling ratio with medium pH for (a) HEMA–VPBA–DMAPM (HBAp) and (b) HEMA–VPBA–DMAEM (HBAe) terpolymer gels.

ligand (usually *m*-aminophenylboronic acid).[1–9] Then, the boronate gels obtained by such a two-step procedure were tried in the affinity chromatography applications of various nucleotides.[1–6] In our study the incorporation of the boronic acid unit during the gel preparation allowed the direct use of gel as a specific sorbent without applying an additional activation procedure. First, the variation of model nucleotide (β-NAD) adsorption capacity of the HBAp gel with medium pH was examined. For this purpose, the gel sample prepared with the VPBA and DMAPM feed concentrations of 2.6 and 20.4 mol % was used as a sorbent. The adsorption experiments were conducted under the conditions described in the experimental section. The variation of equilibrium adsorption capacity with medium pH is given in Figure 3. As seen here, the maximum equilibrium adsorption capacities were obtained in the pH range of 8.5–9.0. This range, which was very close to the pKa value of a phenylboronic acid (pH 8.6), was also consistent with the results reported in the literature.[5–6] Then a pH of 8.5 was selected as an appropriate value for using in the other adsorption experiments. For the derivation of a sample β-NAD adsorption isotherm, the gel prepared with the VPBA and DMAPMA feed ratios of 2.6 and 34.0 mol % was utilized as the sorbent. The initial β-NAD concentration was changed in the range of 0.01–0.56 mg/mL. The adsorption isotherm for β-NAD is given in Figure 4. As seen here, the plateau value of equilibrium adsorption capacity was obtained after the equilibrium β-NAD concentration of 0.4 mg/mL. The plateau value of the equilibrium adsorption capacity was approximately 33.0 mg β-NAD/g dry gel. The equivalent value based on the wet gel was calculated as 8.03 μmol β-NAD/mL. In the literature β-NAD adsorption capacities in the range of 5–7 μmol β-NAD/mL wet gel were reported for the agarose-based boronate gels prepared in particle form.[6] Although HBAp gel was used in the form of a single tablet in the adsorption experiments, the

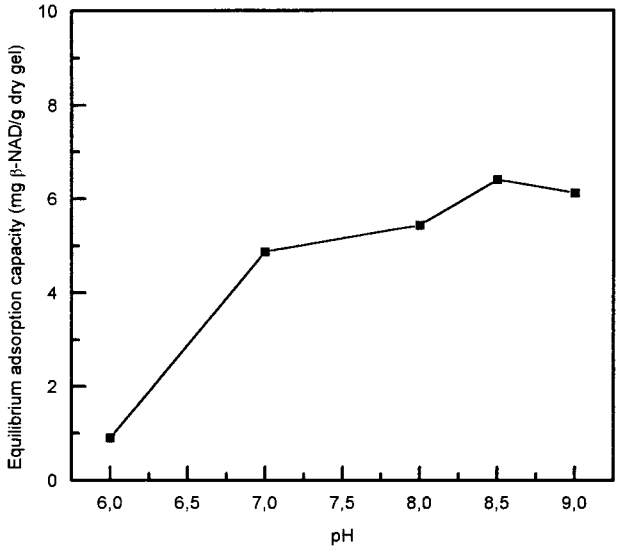

Figure 3 The effect of pH on equilibrium β-NAD adsorption capacity of boronic acid–functionalized HEMA gel.

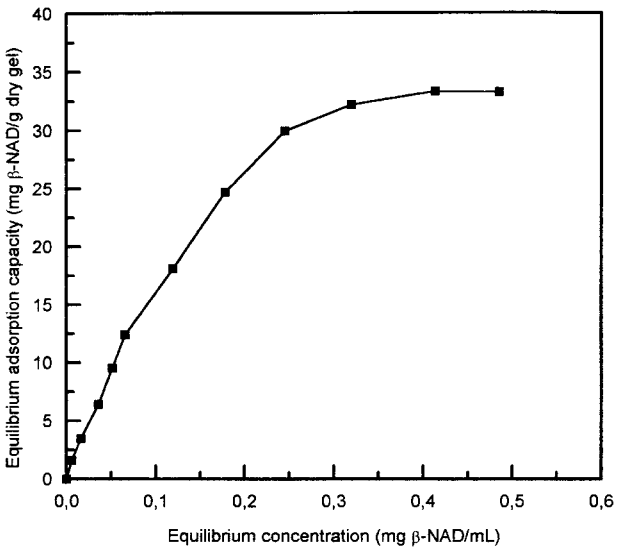

Figure 4 The adsorption isotherm for β-NAD obtained by using HBAp gel produced by the VPBA feed concentration of 2.6 mol % as a sorbent.

adsorption capacities reported for the sorbents in the particulate form could be achieved with the proposed material. The adsorption isotherm can be described by the Langmuir model

$$q = q_{\max} n C_{\text{eq}} / (1 + n C_{\text{eq}}) \qquad (2)$$

where q is the equilibrium β-NAD concentration in the gel matrix (mg β-NAD/g dry gel), C_{eq} is the equilibrium β-NAD concentration in solution (mg β-NAD/mL), and n and $q_{\max}$ are the Langmuir constant (mL/mg β-NAD) and the maximum adsorption capacity (mg β-NAD/g dry gel), respectively. Based on the linearized form of this equation, the plot of $1/C_{eq}$ versus $1/q$ was employed to obtain the intercept of $1/q_{\max}$ and the slope of $1/q_{\max} n$.

$$1/q = [1/(q_{\max} n)][1/C_{\text{eq}}] + [1/q_{\max}] \qquad (3)$$

By applying a least-squares algorithm, the following values were obtained:

$$1/q_{\max} = 0.02845 \pm 0.00578$$

$$1/q_{\max} n = 0.00364 \pm 0.00010$$

$$r^2 = 0.99258$$

From these values, $q_{\max}$ and n were calculated as 35.2 mg β-NAD/g dry gel and 7.81 mL/mg β-NAD, respectively.

The desorption ratios obtained by applying the procedure in the experimental section are given in Table I. As seen here, the adsorbed β-NAD could be effectively desorbed from the sorbent, with the yields varying between 93.2 and 98.1.

Effect of VPBA Feed Concentration

A series of gels were prepared by changing the VPBA feed concentration to be between 0 and 6.1 mol %. In the preparation of these gels, DMAPM feed concentration was fixed at 20.4 mol %. The equilibrium swelling behavior of these gels is given in Figure 5. As seen here, the increase in the VPBA feed concentration caused a decrease in the equilibrium swelling ratio, both in the acidic and the alkaline pH region. For all samples the swelling ratio of gel decreased with increasing pH because of the decreasing ionization degree of dimethylamino groups. The gels prepared with different VPBA feed concentrations were used as sorbents in the β-NAD adsorption experiments. First, the β-NAD adsorption onto the produced gels was investigated dynamically. In these experiments the β-NAD initial concentration in the adsorption medium was fixed at 0.05 mg/mL. The other conditions were the same as those used for the derivation of β-NAD adsorption isotherm. The variation of β-NAD adsorption capacity with time is given in Figure 6. As seen here, higher adsorption rates and higher equilibrium adsorption capacities were obtained with gels prepared with higher VPBA feed concentrations. The increase in

Table I Desorption Yields of β-NAD-Adsorbed Gel Matrices Obtained with Different β-NAD Initial Concentrations in Adsorption Medium

β-NAD Initial Concentration (mg/mL)	Desorption Yield (wt %)
0.01	93.2
0.025	96.3
0.05	97.2
0.075	98.1
0.10	97.1
0.16	97.2
0.24	97.1
0.32	96.2
0.40	95.1
0.48	94.1

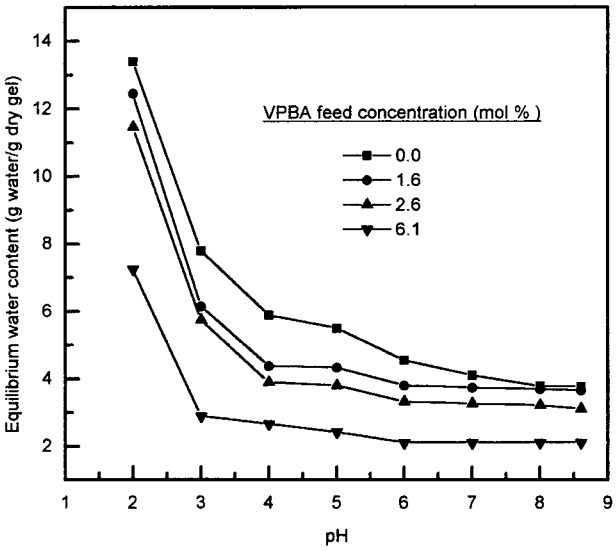

Figure 5 The equilibrium swelling behaviors of the gels produced with different VPBA feed concentrations. DMAPM feed concentration: 20.4 mol %.

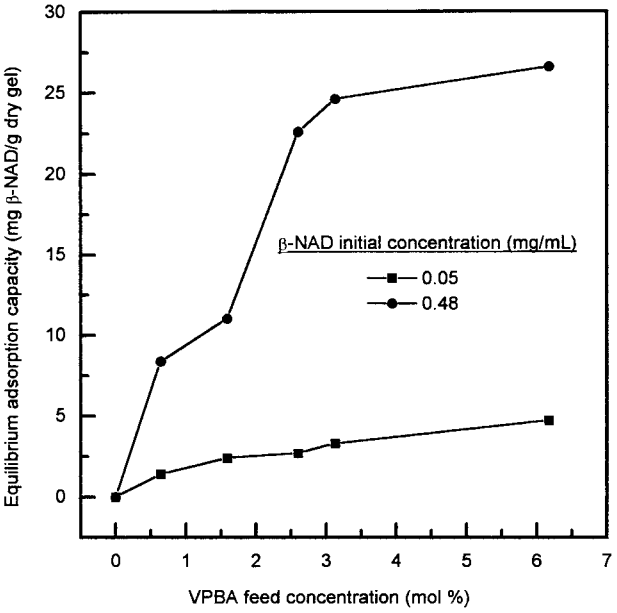

Figure 7 The variation of equilibrium β-NAD adsorption capacity with the VPBA feed concentration for HBAp gels (DMAPM feed concentration: 20.4 mol %).

the equilibrium adsorption capacity was an expected result because a higher number of boronic acid groups were incorporated into the gel structure with increasing VPBA feed concentration. The increase in adsorption rate with increasing VPBA feed concentration was an interesting result because the gels exhibited lower swelling ratios with increasing VPBA feed concentration (Fig. 5). The decrease in the swelling ratio involves a decrease in the micropore volume of the gel, providing an increase in the internal mass transfer resistance. Then a decrease in the diffusion rate of β-NAD molecules is expected. Based on experimental results, it can be concluded that the diffusion of β-NAD probably is not influenced by the decrease in the gel microporosity because it is a reasonably small molecule (M_w: 663.4).

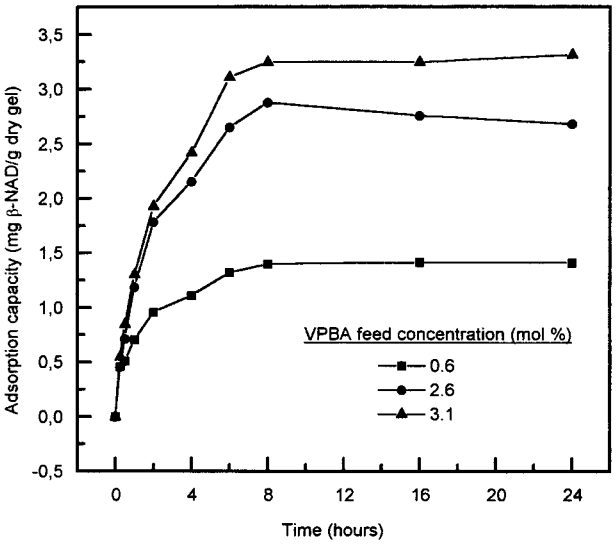

Figure 6 The variation of β-NAD adsorption capacity with the time for HBAp gels produced with different VPBA feed concentrations and by fixing the DMAPM feed concentration to 20.4 mol %. β-NAD initial concentration: 0.05 mg/mL.

The variation in equilibrium β-NAD adsorption capacity with the VPBA feed concentration is shown in Figure 7 for gels prepared with a constant DMAPM feed concentration of 20.4 mol %. In these experiments two β-NAD initial concentrations were utilized. The higher initial concentration (0.48 mg/mL) was selected to observe the tendency in the plateau region of the adsorption isotherm (Fig. 4). The lower initial concentration (0.05 mg/mL) was selected in a region used in the previously published studies on β-NAD adsorption.[5] The other adsorption conditions were the same as those used in the derivation of the β-NAD adsorption isotherm. As expected, a clear increase was obtained in the equilibrium adsorption capacity with increasing VPBA feed concentration for both β-NAD initial concentrations (Fig. 7).

As a reference, the β-NAD adsorption onto the HEMA–VPBA copolymer gels prepared with different VPBA contents in the absence of an amine-

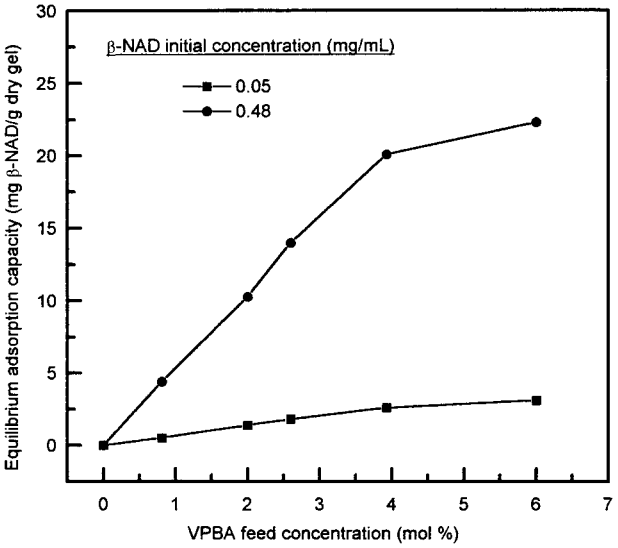

Figure 8 The variation of β-NAD adsorption capacity with VPBA feed concentration for the HEMA–VPBA copolymer gels prepared in the absence of DMAPM.

carrying monomer (i.e., DMAPM) was also investigated. In the gel preparations VPBA feed concentration was varied between 0 and 6.0 mol %. The β-NAD adsorption experiments were conducted under identical conditions as those used in the previous set. Variations of equilibrium adsorption capacity of HEMA–VPBA copolymer gels with the VPBA feed concentration are given in Figure 8. As can be seen, the adsorption capacity increased linearly with increasing VPBA concentration. However, the comparison of adsorption capacities given in Figures 7 and 8 indicates that DMAPM-containing gels exhibited higher β-NAD adsorption capacities relative to the HEMA–VPBA copolymers under identical conditions. Then the effect of an amine-containing monomer on the β-NAD adsorption capacity of the produced gels was examined in detail.

Effect of Amine-Containing Monomer Feed Concentration

First, the effect of amine-containing monomer concentration on the equilibrium swelling behavior of gel was determined. For this purpose, a series of gels was prepared by changing the DMAPM feed concentration to be in the range of 0–47.4 mol %. In these preparations VPBA feed concentration was fixed to 2.6 mol %. For the gels prepared with different DMAPM feed concentrations, the variation of equilibrium swelling ratio with pH is given in Figure 9. As can be seen, the gels prepared with higher DMAPM concentrations exhibited higher equilibrium swelling ratios, both in the acidic and the alkaline pH regions. It should be noted that the swelling curves obtained with the gels prepared in the presence of the other amine-containing monomer (DMAEM) were very similar. First, the effect of feed concentration of an amine-containing monomer on the dynamic adsorption behavior of gel was investigated. In these experiments the initial β-NAD concentration was fixed to 0.05 mg/mL. The variation of adsorption capacity with time is exemplified in Figure 10 for the gels produced with different DMAPM feed concentrations and by fixing the VPBA feed concentration to 2.6. mol %. As can be seen, higher adsorption rates were observed for the gels produced with higher DMAPM feed concentrations. As is known, the specific adsorption of a small biomolecule by a gel matrix may be considered as a two-step process. In the first step the biomolecule diffuses into the gel; then it is adsorbed within because of a specific chemical interaction. The apparent adsorption rate is controlled by one of these steps depending on the magnitude of resistance of each step. The results in Figures 5 and 6 show that the decrease in gel swelling ratio (i.e., possible internal diffusion limitation) was not important for controlling the apparent adsorption rate. Therefore, the increase in the apparent adsorption rate observed in Figure

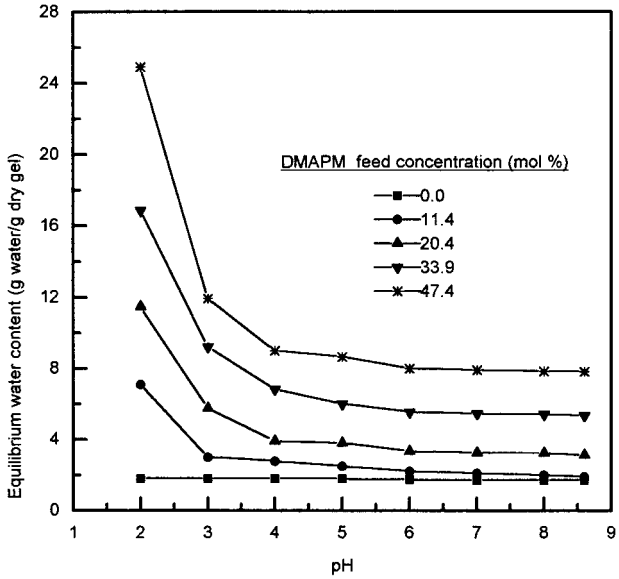

Figure 9 The variation of equilibrium swelling ratio with pH for HBAp gels prepared with different DMAPM feed concentrations (VPBA feed concentration: 2.6 mol %).

10 is not explained by the increase that occurred in gel swelling from the increasing DMAPM concentration (i.e., decreasing internal diffusion resistance; Fig. 9). The only reason that explains the increase observed in Figure 10 may be the increase that occurred in the rate of complex formation (i.e., chemical interaction between the boronate groups of gel and the diol groups of β-NAD) by the increasing DMAPM concentration. The effect of feed concentration of an amine-containing comonomer on the equilibrium β-NAD adsorption capacity of gels was investigated by using gels prepared with different DMAPM and DMAEM feed concentrations and by fixing the VPBA feed concentration to 2.6 mol %. The variation of equilibrium β-NAD adsorption capacity with the feed concentration of an amine-containing monomer is given in Figure 11. Here the adsorption experiments were performed with two different β-NAD initial concentrations under the conditions used for the derivation of β-NAD adsorption isotherm. As seen in Figure 11(a), the equilibrium adsorption capacity increased with the increasing DMAPM feed concentration for both initial concentrations of β-NAD. As explained in the literature, the diol-binding affinity of boronate gels was increased by the incorporation of an amine group into the gel structure.[14,15] This result was reported for the binding of diol-containing compounds (i.e., polyvinyl alcohol and glucose) to the

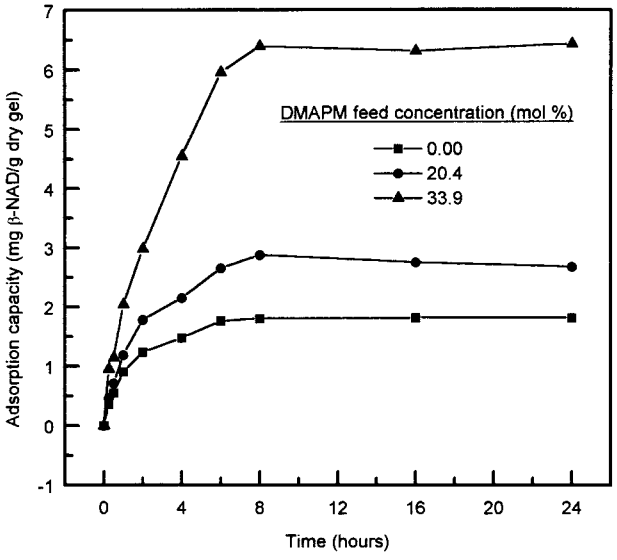

Figure 10 The variation of β-NAD adsorption capacity with the time for the gels prepared with different DMAPM feed concentrations. VPBA concentration: 2.6 mol %.

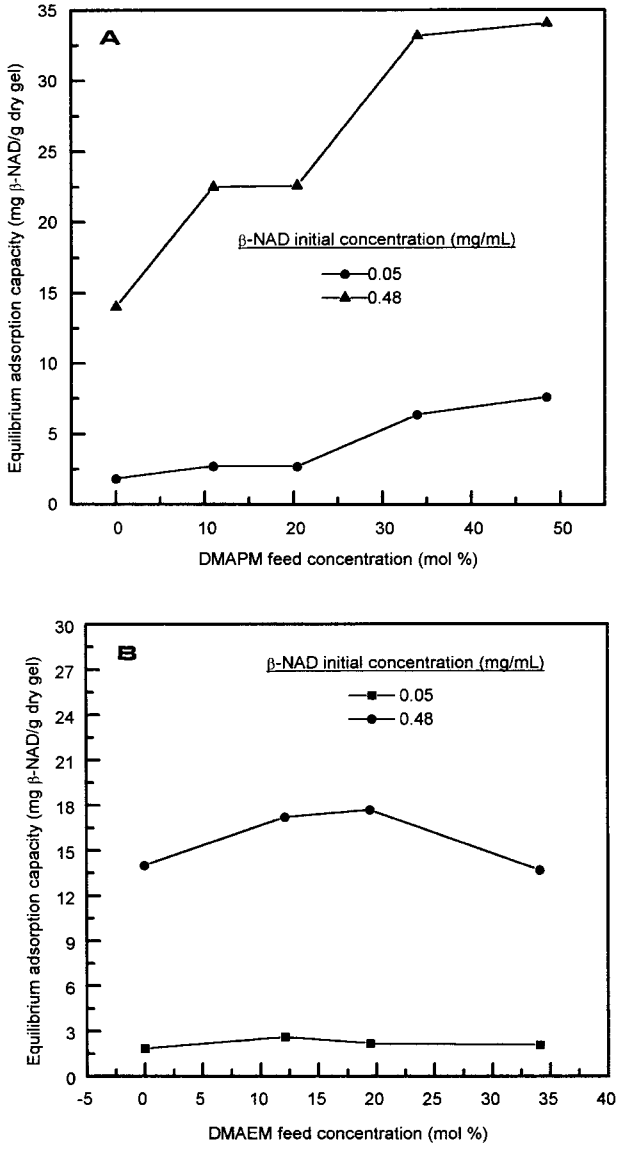

Figure 11 The variation of equilibrium β-NAD adsorption capacity of terpolymer gels with the feed concentration of amine-containing monomer; amine-containing monomer types: (a) DMAPM, (b) DMAEM.

soluble terpolymers of *m*-acrylamidophenylboronic acid, *N,N*-(dimethylamino)propylacrylamide and *N,N*-dimethylacrylamide.[14,15] Our results indicated that this conclusion was valid for the binding of nucleotides to the HEMA-based boronate gels when DMAPM was included as the comonomer in the gel preparation. However, the effect of feed concentration of other amino-group-containing monomers on nucleotide adsorption capacity was different. The variation of β-NAD adsorption capacity of HBAe gels with DMAEM feed concentration is given in Figure 11(b). Al-

though the effect of DMAEM concentration on equilibrium adsorption capacity is not so clear in this figure, it is evident that DMAEM was not as effective as DMAPM in increasing β-NAD adsorption capacity. To explain the result, the structures of both monomers are included below:

DMAPM

$$H_2C{=}C(CH_3)CO{-}NH{-}(CH_2)_3N(CH_3)_2$$

DMAEM

$$H_2C{=}C(CH_3)CO{-}O{-}(CH_2)_2{-}N(CH_3)_2$$

Although an increase in nucleotide-binding ability was observed in the presence of the amide group —NH—$(CH_2)_3N(CH_3)_2$, the introduction of the ester —O—$(CH_2)_2$—$N(CH_3)_2$ reduced the nucleotide adsorption. By comparing the variations in Figure 11(a,b) it can be concluded that the presence of a dimethylamino group is not only sufficient to increase the nucleotide-binding affinity of boronic-acid-carrying gels—it seems that the whole structure carrying the amino group is probably responsible for the increasing interaction between boronic acid groups of the gel and diol groups of the biomolecule.

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