Ion Imprinted Beads Embedded Cryogels for *In Vitro* Removal of Iron from β-Thalassemic Human Plasma

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ABSTRACT: Molecular recognition based Fe^{3+} imprinted poly(GMA-MAC) (MIP) beads embedded PHEMA composite cryogel was prepared for selective removal of Fe^{3+} ions from β-thalassemia patient plasma. The precomplexation was achieved by the coordination of Fe^{3+} ions with *N*-methacryloyl-(L)-cysteine methyl ester (MAC-Fe^{3+}). MIP beads were prepared by dispersion polymerization in the presence of MAC-Fe^{3+} complex and glycidyl methacrylate (GMA) monomer. Then the MIP beads were embedded into poly(hydroxyethyl methacrylate) (PHEMA) cryogel. The specific surface area and the swelling degree of the PHEMA-MIP composite cryogel were found to be 76.8 m^2/g and 7.7 g H_2O/g cryogel, respectively. The maximum adsorption amount of Fe^{3+} ions was 2.23 mg/g. The relative selectivity of PHEMA-MIP composite cryogel towards the Fe^{3+} ions was 135.0, 61.4, and 57.0 times greater than that of the PHEMA-NIP cryogel as compared with the Ni^{2+}, Zn^{2+}, and Fe^{2+} ions, respectively. PHEMA-MIP composite cryogel was recovered and reused many times without any significant decrease in its adsorption capacity. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 125: 254–262, 2012

Key words: iron removal; **β**-thalassemia; ion imprinting; composite cryogels

INTRODUCTION

Iron is an essential element for the proper functioning of all living cells, for processes such as oxygen transport, electron transfer, and DNA synthesis.[1] Although its importance, it is toxic when present in excess. In the presence of molecular oxygen, free iron is able to catalyze the formation of free hydroxyl radicals, which are quite harmful for biomolecules including sugars, lipids, proteins, and nucleic acids, resulting in peroxidative tissue damage.[2]

Iron overload is the most serious complication of β-thalassemia and is the main point of its management.[3] Also in patients with hemochromatosis, abnormal iron absorption leads to a 2–5 g increase in the body iron level per year.[4] Regular blood transfusions lead to double this iron accumulation. Human cannot actively eliminate iron from the body once it has been acquired.[4] Iron chelation therapy has been shown to reduce iron-related morbidity and to improve quality of life in patients with β-thalassemia.[5] Desferrioxamine-B is the most widely used iron chelator over the past 30 years. However, it has some drawbacks in its application due to ocular, auditory, and renal disturbances and it becomes highly toxic at high doses or over prolonged periods of time.[6]

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To overcome the adverse effects of soluble iron chelators, the attachment of iron chelating ligands including dye and protein molecules has been studied.[7,8] Compared with the soluble iron chelators, iron chelating resins have some advantages in terms of stability, reusability, and minimal damage to biomolecules. Developing an efficient iron chelating system for the treatment of the patients with chronic iron overload usually involves the preparation of suitable and effective adsorbents to excrete the iron in the body. For instance, molecular imprinted polymers (MIP) have recently gained a close attention as one of the most promising adsorbents.[9–13] However, there have been only a few reports of MIPs developed for the *in vitro* removal of iron out of human plasma with severe iron overload.[14–17]

The need for fast separation processes to achieve these requirements has driven the evolution of different types of chromatographic support materials.[18] Cryogels are very good alternative adsorbents for bioseparation with many advantages such as very large pores, short diffusion path, low pressure drop, and very short residence time for both adsorption and elution.[19] Cryogels are also cheap materials and they can be used as disposable avoiding cross-contamination between batches. They can be used as a monolithic form as well as in a membrane form to combine the advantage of membrane chromatography, in terms of high flow-rates and high productivity.[20] Short diffusional distances allow optimal utilization of the immobilized ligand on the pore walls.[20–23] The disadvantage

of the cryogels, on the other hand, is their low surface area and thus low adsorption capacity.[24–26] In actual, bioseparation processes, it is a great importance to increase the adsorption capacity of supermacroporous cryogel. Therefore, bead embedding would be a useful improvement mode to use in the preparation of novel composite cryogels for increasing both surface area and adsorption capacity.[27–30]

Here, we reported the development and application of Fe^{3+} ion imprinted poly(GMA-MAC) beads embedded PHEMA composite cryogel for the *in vitro* removal of Fe^{3+} ions from thalassemic human serum.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA), 2-hydroxyethyl methacrylate (HEMA), *N*,*N*′-methylene-bis(acrylamide) (MBAAm), azobisisobutyronitrile (AIBN), poly(vinyl pyrrolidone) (PVP) (M_W: 58,000), L-Cysteine methylester and methacryloyl chloride were obtained from Sigma Chemical (St. Louis, MO). *N*,*N*,*N*′,*N*′-tetramethylene diamine (TEMED) was supplied by Fluka A.G. (Buchs, Switzerland). All other chemicals were of reagent grade and purchased from Merck AG (Darmstadt, Germany). All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion exchange packed-bed system.

Synthesis of *N*-methacryloyl-(L)-cysteinemethylester

The preparation and characterization of the *N*-methacryloly-(L)-cysteinemethylester (MAC) is given in detail elsewhere.[14] Briefly, the following experimental procedure was applied for the synthesis of MAC monomer: 5.0 g of L-cysteine methylester and 0.2 g of $NaNO_2$ were dissolved in 30 mL of K_2CO_3 aqueous solution (5%, v/v). The solution was cooled down to 0°C followed by a slow addition of 4.0 mL of methacryloyl chloride under nitrogen atmosphere and then stirred magnetically at room temperature for 2 h. At the end of the reaction, the pH of the reaction mixture was adjusted to 7.0 which were then extracted with ethyl acetate. The aqueous phase was evaporated under vacuum to yield the desired product. The desired product residue (MAC) was crystallized in a mixture of ethanol and ethyl acetate.

Preparation of the complex monomer (MAC-Fe^{3+})

The complex monomer, MAC-Fe^{3+}, was prepared by dissolving solid *N*-methacryloyl-(L)-cysteinemethylester (MAC) (0.19 g, 1.0 mmol) in 0.5 mL water (pH: 3.0) in an eppendorf tube. Totally, 0.202 g of iron salt ($Fe(NO_3)_2.9H_2O$) (0.5 mmol) was also dissolved in water at room temperature. Then, two solutions were mixed and the mixture was gently stirred to allow formation of the stable complex.

Preparation of MIP beads

The dispersion polymerization was performed in a glass reaction vessel (250 mL) equipped with an anchor-type stirrer (760 rpm) and a temperature control system. A typical procedure applied for the dispersion polymerization is given in detail elsewhere.[31] Briefly, 4 g of PVP was dissolved in a dispersion medium containing 84 mL ethanol in reaction flask. Monomer phase was composed of 9.5 mL GMA, 0.2 mg AIBN, 0.1 mL EGDMA, and 9 mL toluene as a porogen. Fe^{3+}-MAC complex was added into homogenous monomer phase. Then the two phases were gathered and sonicated for about 5 min at 200 W with an ultrasonic water bath (Bransonic 2200, England). After purging with nitrogen gas for 20 min, the flask was immersed into the preheated water bath at 50°C while stirring at 680 rpm. The initial polymerization time was defined when the reactor temperature was raised to 70°C. The overall polymerization time was 14 h. After completion of the polymerization period, the reactor content was cooled down to room temperature and centrifuged at 7500 rpm for 10 min for the removal of dispersion medium. Fe^{3+} imprinted poly(GMA-MAC) (MIP) beads were redispersed within 10 mL of ethanol/water mixture and centrifuged again under similar conditions. The ethanol/water washing was repeated ten times for complete removal of unconverted monomers and other components. Finally, MIP beads were washed extensively with water, dried in oven and stored at room temperature. Nonimprinted poly(GMA-MAC) (NIP) beads were prepared by the same procedure without adding Fe^{3+} ions into polymerization mixture.

Preparation of PHEMA-MIP composite cryogel

Preparation of the PHEMA cryogel with embedded MIP beads is described below. Briefly, monomers (1.3 mL HEMA and 0.275 g *N*,*N*′-methylene-bis(acrylamide) (MBAAm)) were dissolved in deionized water (10 mL) and MIP beads were also swelled in deionized water (5 mL). Then, MIP beads solution was added into the monomer solution. The mixture was degassed under vacuum for about 5 min to eliminate soluble oxygen. Total concentration of monomers was 12% (w/v). The cryogel was produced by free radical polymerization initiated by *N*,*N*,*N*′,*N*′ tetramethylene diamine (TEMED, 25 μL)

and ammonium persulfate (APS, 20 mg). After adding APS (1% (w/v) of the total monomers) the solution was cooled in an ice bath for 2–3 min. TEMED (1% (w/v) of the total monomers) was added and the reaction mixture was stirred for 1 min. The amount of embedded beads was 300 mg. The reaction mixture was poured into a plastic syringe (5 mL, id. 0.8 cm) with closed outlet at the bottom. The polymerization solution in the syringe was frozen at −16°C for 24 h and then thawed at room temperature. After washing with 200 mL of water, the PHEMA-MIP composite cryogel was washed extensively with 50 m*M* EDTA solution for the removal of template (i.e., iron) molecules from the polymeric structure. For this purpose, elution solution was passed through the PHEMA-MIP composite cryogel at room temperature for 24 h. This procedure was repeated until no Fe^{3+} leakage was observed from the polymeric structure to the wash solution. The iron free cryogel was cleaned with ethanol and water at room temperature for 12 h. PHEMA-NIP composite cryogels were prepared by embedding NIP beads into PHEMA cryogel.

Characterization studies

Porosity of the PHEMA cryogel, MIP beads and PHEMA-MIP composite cryogel were measured by a N_2 gas sorption technique, performed on Flowsorb II, (Micromeritics Instruments Corporation, Norcross, USA). The specific surface areas of polymers in dry state were determined by multipoint Brunauer-Emmett-Teller (BET) apparatus (Quantachrome, Nova 2200E, USA). 0.5 g of samples were placed in sample holder of BET and degassed by passing through N_2-gas at 150°C for 1 h. The adsorption of N_2 gas was performed at −210°C while its desorption was performed at room temperature. Experimental values obtained from desorption step was used to calculate the specific surface area of the polymer sample. Pore volume and an average pore diameter for sample were determined by the method on a BJH (Barrett, Joyner, Halenda) model.

Water uptake ratio of the polymers was determined using distilled water. The water uptake experiments were conducted as follows: dry sample (PHEMA and PHEMA-MIP cryogels) was carefully weighed out before being soaked into 50 mL vial containing distilled water. The vial was then placed into an isothermal water bath at 25°C for 2 h, after which the wet-polymer sample was taken out of the vial, wiped out with a filter paper, and weighed. The weight ratio of dry and wet sample was recorded. The water content of the PHEMA and the PHEMA-MIP cryogel was calculated as $[(W_s - W_o)/W_o] \times 100$, where W_o and W_s are the weights of polymers before and after uptake of water. The total volume of macropores in the swollen cryogel was roughly estimated by weighing the sample ($m_{\text{squeezed gel}}$) after squeezing the free water from the swollen gel matrix, then the porosity was calculated as $(m_{\text{swollen gel}} - m_{\text{squeezed gel}})/m_{\text{swollen gel}} \times 100\%$. The percent swollen gel weight is also calculated as $(m_{\text{swollen gel}} - m_{\text{dried}})/m_{\text{swollen gel}} \times 100\%$. The flow rate of water passing through the column was measured at the constant hydrostatic pressure equal to 100 cm of water-column corresponding to a pressure of ca. 0.01 MPa. All the measurements were replicated at least three times.

The surface structure of the PHEMA cryogel, MIP beads and PHEMA-MIP composite cryogel was visualized and examined by scanning electron microscopy (SEM). MIP beads were dried at 25°C for seven days and PHEMA cryogel and PHEMA-MIP composite cryogel were freeze dried in order to protect pore structures. After the polymer samples were dried, tiny fragments of the polymers were mounted on SEM sample holders on which they were sputter coated for 2 min. The samples were then consecutively mounted in a scanning electron microscope (Model: Jeol JEM 1200 EX, Tokyo, Japan) to visualize the surface structures of each polymers at desired magnification levels.

The FTIR spectroscopy was implemented on a Thermo Fisher Scientific, Nicolet iS10 (Waltham, MA, USA) machine to assign the functional groups detected in the wavenumber range of 4000–450 cm^{-1} for the MAC monomer, MAC-Fe^{3+} complex and MIP beads samples in the solid state.

Blood-compatibility studies

The interaction of the human blood with PHEMA and PHEMA-MIP composite cryogel was monitored by measuring the following parameters; prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen time and thrombocyte clotting time. The *in vitro* hemocompatibility tests were implemented by STA4 Compact Blood Coagulation Analyzer (Diagnostica Stago, France) using the required test kits.

Fe^{3+} removal from β-thalassemia human plasma

The Fe^{3+} removal studies from β-thalassemia human plasma were preformed with PHEMA-NIP and the PHEMA-MIP composite cryogel columns. 50 mL aliquots of the blood sample were centrifuged at 500 g for 30 min at room temperature. The cryogel column, equipped with a water jacket for temperature control was degassed under reduced pressure by a water suction pump. The composite cryogel column was then equilibrated by passing through four column volumes of phosphate buffer adjusted at pH: 7.4. Then, 10 mL of the plasma with β-thalassemia was passed through

the composite cryogel column at moderate pressure by a peristaltic pump over 2 h. The Fe^{3+} ions captured by the composite cryogel column were eluted by a solution of 0.05 M EDTA. The concentration of Fe^{3+} ions in the eluted phase was determined by a graphite furnace atomic absorption (GFAA) spectrophotometer and the amount of Fe^{3+} ions removed was calculated by using a mass balance approach.

Selectivity experiments

The specificity of PHEMA-MIP and PHEMA-NIP composite cryogels towards Fe^{3+} ions was assessed by competitive adsorption studies in the presence of Fe^{3+}, Ni^{2+}, and Zn^{2+} ions and also in the presence of Fe^{3+} and Fe^{2+} ions. To accomplish the competitive adsorption studies, 10 mL of Fe^{3+} (5 ppm), Zn^{2+} (5 ppm), Ni^{2+} (5 ppm), and 10 mL of Fe^{3+} (5 ppm) and Fe^{2+} (5 ppm) cation spiked plasma were used. The ions were determined by Bio-LC (Dionex, USA) with Ion Pac CG5A precolumn, CS5A analytical column and diode array detector (PDA-100). The mobile phase was PAC PDCA solution (water: 7 m*M* PDCA: 66 mM KOH: 5.6 m*M* K_2SO_4: 74 m*M* formic acid in the ratio of 95.8 : 0.58 : 1.85 : 0.49 : 1.70 w/v %). Injection volume was 50 μL and the measurement wavelength was 530 nm.

Distribution coefficients of (K_d) for Zn^{2+}, Ni^{2+}, and Fe^{2+} with respect to Fe^{3+} were calculated by eq. (1).[32]

$$K_d = [(C_i - C_f)/C_f] \times V/m \quad (1)$$

where K_d (mL/g) is a distribution coefficient for the competing metal ion. C_i and C_f are the initial and the final concentrations of metal ions (μg/mL), respectively. V is the volume of the mixture (mL) and m is the mass of adsorbent (g).

The generic term in eq. (2) was adopted to determine a selectivity coefficient (k) for the binding of Fe^{3+} onto the composite cryogel in the presence of a competing species such as Zn^{2+} and Ni^{2+}, in which K_d (competing metal ion) is the distribution coefficient of the competing metal ion.

$$k = K_{d\ (\text{template metal ion})}/K_{d\ (\text{competing metal ion})} \quad (2)$$

A ratio of the selectivity coefficients (k') in eq. (3), in which $k_{\text{imprinted}}$ is for the Fe^{3+} imprinted and k_{control} is for the PHEMA-NIP in the presence of the Fe^{3+}-Zn^{2+}-Ni^{2+} ions was used as an approximation to assess the effect of imprinting on ion selectivity.

$$k' = k_{\text{imprinted}}/k_{\text{control}} \quad (3)$$

Desorption and reuse

Fe^{3+} bound to PHEMA-MIP composite cryogel is desorbed by recirculating 0.05 M EDTA through the column. In a typical desorption procedure, 20 mL of desorption agent was recirculated through the PHEMA-MIP composite column at room temperature for 1 h. The final Fe^{3+} concentration in desorption medium was determined by GFAA spectroscopy, the result of which was then converted to a total amount of Fe^{3+} (mg) in the desorbed media (M_d) giving rise to the amount of desorbed Fe^{3+} per gram of column weight. The initial amount of Fe^{3+} adsorbed on the PHEMA-MIP composite column (M_i) was also determined in terms of mg Fe^{3+}/g column. The percent desorption of Fe^{3+} was then determined by the eq. (4);

$$\%\ \text{desorption} = [(M_d/M_i) \times 100] \quad (4)$$

In order to evaluate the reusability of the PHEMA-MIP composite column, the Fe^{3+} adsorption–desorption cycle was repeated 20 times with the same column that was rinsed with a solution of 50 m*M* NaOH after each desorption procedure to ensure sterility.

TABLE I
Physical Properties of Adsorbent

	Polymer	
	PHEMA cryogel	Poly(GMA-MAC)/ PHEMA
Surface Area (m^2/g)	11.7	76.8
Swelling Degree (g H_2O/g polymer)	8.3	7.7
Macroporosity (%)	70	66
MAC incorporation (μmol/g)	–	192.8
Flow Resistance (cm/h)	545	845

RESULTS AND DISCUSSION

Characterization studies

The PHEMA-MIP composite cryogel was produced by polymerization in the frozen state of HEMA and MBAAm in the presence of MIP beads. Some properties of the PHEMA cryogels and the PHEMA-MIP composite cryogels were summarized in Table I. The specific surface area of PHEMA-MIP composite cryogels was determined as 76.8 m^2/g by a multipoint BET apparatus. As can be seen from Table I, the embedding of MIP beads resulted in about seven fold increase in surface area of the PHEMA cryogels. The scanning electron micrographs of the MIP beads, nonembedded and bead embedded cryogels are shown in Figure 1(A,B,C), respectively. The macropores in the cryogel structure are open and highly interconnected, forming a network of large channels. The mobile phase (i.e., blood plasma) is forced to flow through them, transporting the Fe^{3+} ions to the

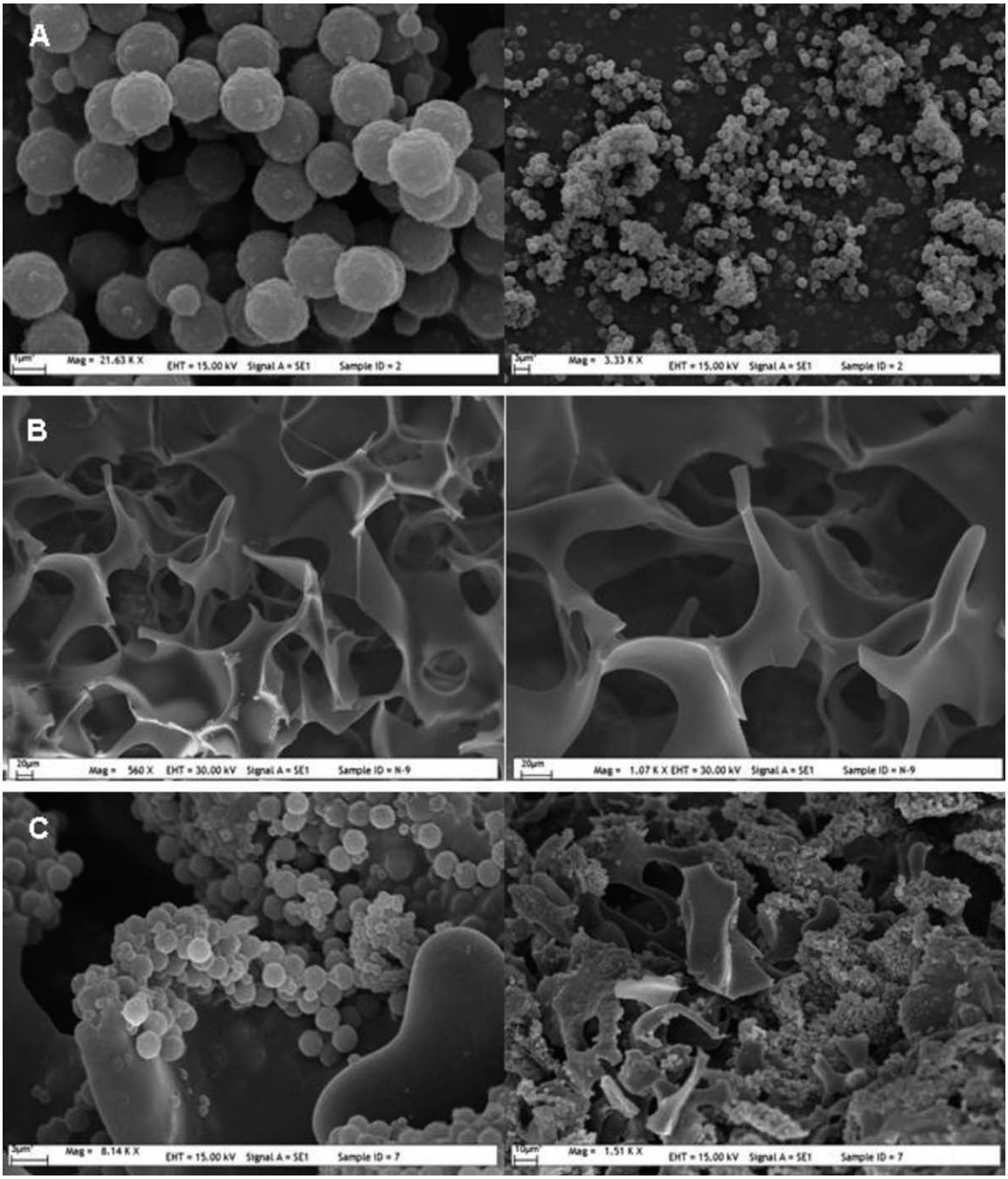

Figure 1 Scanning electron photographs of (A) MIP beads (B) PHEMA and (C) PHEMA-MIP cryogels.

specific cavities by convection. This results in an extremely fast mass exchange between the mobile phase and the stationary cryogel phase then the more traditional packed bed columns.[33,34] The presence of embedded beads can be seen clearly. Pore size of the matrix is much larger than the size of the ions, allowing them to pass easily. As a result of the convective flow of the solution through the pores, the mass transfer resistance is practically negligible. The equilibrium swelling degree was 7.7 g H_2O/g cryogel for the PHEMA-MIP composite cryogel. The equilibrium swelling degree of PHEMA cryogels was 8.3 g H_2O/g. In comparison with the PHEMA cryogel, the equilibrium swelling degree of composite cryogel was decreased due to the incorporation of solid particles. Cryogels are opaque, sponge like and elastic. They can be easily compressed by hand to remove accumulated water inside the pores. When the compressed piece of cryogel was submerged in water, it soaked in water and restored its original shape within 1–2 s. The embedding of the MIP beads also resulted in more mechanically stable

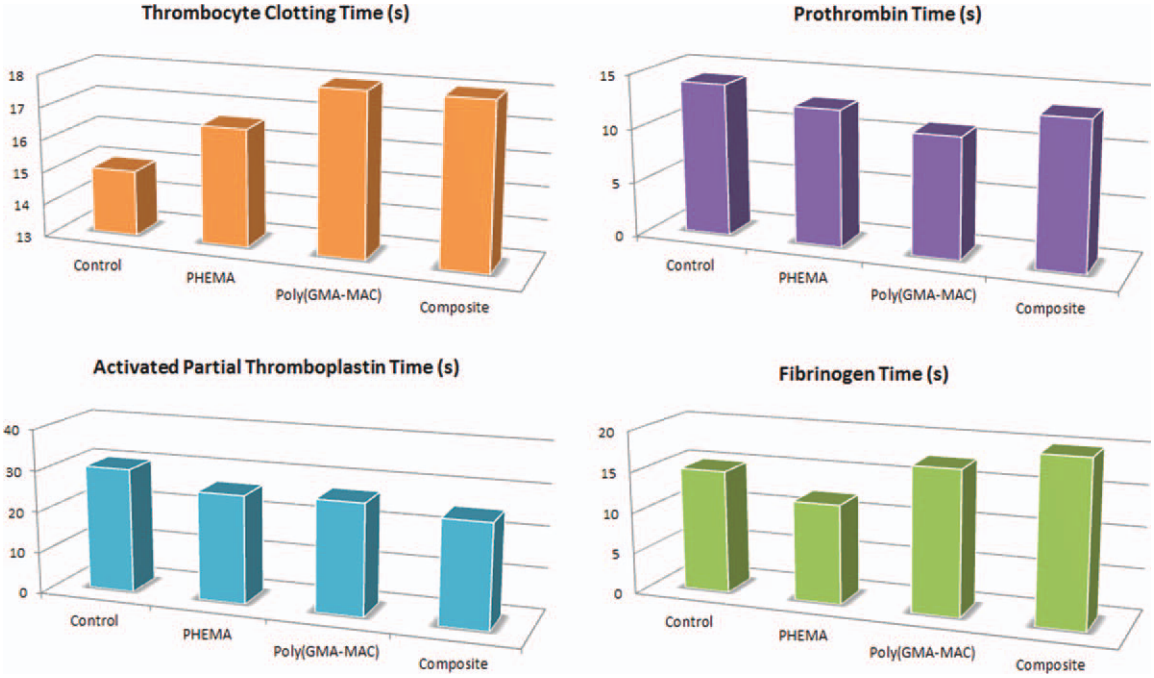

Figure 2 Coagulation times of human plasma (reported in sec). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cryogel systems. It should be noted that there were no MIP beads release in any of the adsorption and desorption media.

To test the blood-compatibility of the composite adsorbent, the *in vitro* fibrinogen time, activated partial thromboplastin time (APTT), prothrombin time (PT), and thrombocyte clotting time was determined by the procedures described in experimental section and the results are outlined in Figure 2. Observed decreases in coagulation times are in the tolerable range of the body.[35] Therefore, it is concluded that the blood-compatibility of the composite cryogels is quite acceptable and the clotting times determined in our laboratory are consistent with the clotting data reported in a relevant literature.[36]

Fe^{3+} Removal from thalassemic human plasma

Fe^{3+} removal experiments from the β-thalassemic human plasma were performed from a series of media containing different amounts of Fe^{3+}. The adsorption results were plotted in an isotherm in Figure 3. The Fe^{3+} adsorption linearly increases as the Fe^{3+} concentration in media is increased up to 90 μg Fe^{3+}/dL, after which any further increment in the Fe^{3+} concentration saturates the active binding cavities on composite cryogel (Fig. 3). The increasing trend in the binding isotherm in Figure 3 confirms the binding cavities formed by the ion-imprinting technique applied in our work. The maximum amount of Fe^{3+} adsorbed by PHEMA-MIP composite cryogel was found to be 2.23 mg/g. It should be noted that the nonspecific binding of Fe^{3+} to PHEMA-NIP cryogel was very low (0.78 mg/g). Metal ion imprinting process has shown marked enhancement of adsorption of corresponding metal ion by creating specific recognition sites into polymeric structure. During the imprinting process, template ions were organized with functional monomer (i.e., MAC) molecules while polymer chains were growing with a memory for both coordination sphere and size of the template ion. So, the higher specific adsorption dynamics would be occurred. In this respect, preorganization of Fe^{3+} ions with MAC monomer gained specific memory to the embedded MIP beads. Also, the faster and the higher Fe^{3+} ion

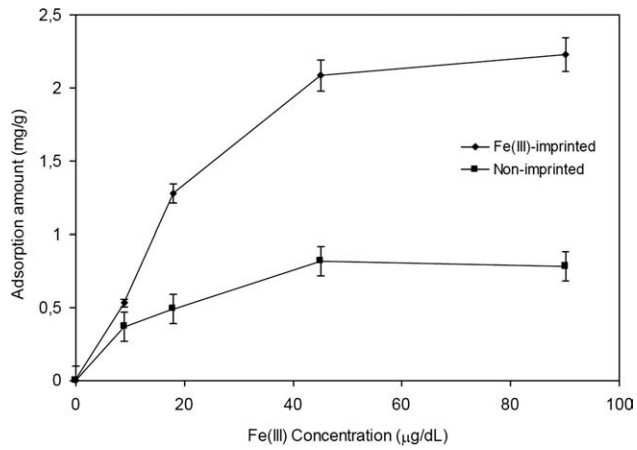

Figure 3 Adsorption isotherm for PHEMA-MIP and PHEMA-NIP composite cryogel at different Fe^{3+} initial concentrations. Flow rate: 0.5 mL/min; T: 25°C.

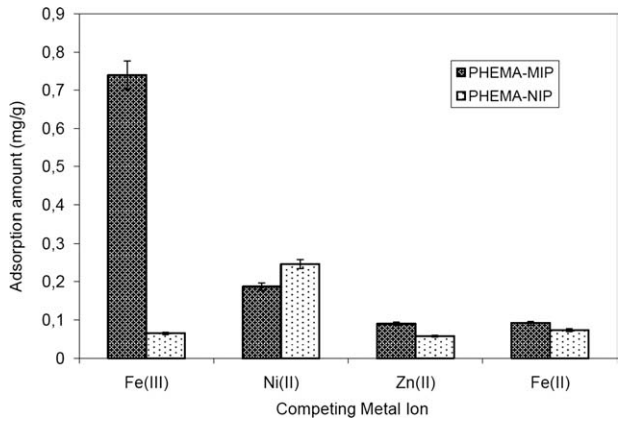

Figure 4 Adsorption of Fe^{3+} ions in the presence of competitor ions Zn^{2+}, Ni^{2+}, and Fe^{2+}.

adsorption capacity of the composite cryogel are not only stemmed from good affinity to recognition site, but also due to smaller diffusion barrier in the highly hydrophilic cryogel. The lower diffusion resistance causes that Fe^{3+} ions can easily go through cryogel network, then reach to specific recognition element (MAC) in MIP structure and bind fast.

There are several attempts to prepare adsorbents for iron ion adsorption. Microporous PHEMA films carrying cibacron blue F3GA, Congo red and ferritin were prepared by Yavuz et al. for iron ion removal from human plasma. The maximum amounts of Fe(III) removed from human plasma by cibacron blue F3GA, Congo red, and ferritin attached PHEMA films were 3.80 μg/cm^2, 4.41 μg/cm^2 and 8.1 μg/cm^2, respectively.[8] Karabörk et al prepared inorgano-organo Fe^{3+} ion imprinted polymer nanocomposite traps using methacryloylamidoantipyrine as functional monomer. Their maximum Fe^{3+} binding capacity was 78.5 mg/g and the selectivity of the adsorbent was 5.28, 11.4, 15.8, and 72.6 times higher with respect to Al^{3+}, Cu^{2+}, $Co^{2,+}$ and Zn^{2+} ions, respectively.[37] Chang et al. prepared Fe(III)-imprinted amino functionalized silica gel and their maximum static adsorption capacity for Fe(III) was 25.21 mg/g with a 49.9 times higher selectivity than Cr(III).[38] Aslıyüce et al. used iron imprinted PHEMAC monolithic cryogels to remove Fe(III) ions from human plasma. Their maximum adsorption capacity was 75 μg/g and the selectivity coefficients of the adsorbent were 12.6 and 2.3 for Cd^{2+} and Ni^{2+}, respectively.[15] Yavuz et al. investigated Fe^{3+} removal performance of Fe^{3+} imprinted poly(HEMA-MAGA) beads[16] and poly(HEMA-MAGA) membranes[17] from iron overdosed human plasma. Their adsorption capacity was 92.6 μmol/g for beads and 164.2 μmol/g for membranes. Karabörk et al. prepared Fe(III) imprinted poly(MAAP-EGDMA) beads with Fe(III) adsorption capacity of 29.32 mg/g.[39] Thermosensitive polymers were also used for the preparation of iron imprinted adsorbents. Utku et al. prepared thermosensitive Fe(III) imprinted poly(NIPA-MAC) particles for Fe(III) removal from human plasma. They reached about 40 mg/g Fe(III) adsorption capacity.[40] Özkara et.al. investigated the performance of Fe^{3+} imprinted PHEMAC monolith for removal of Fe^{3+} ions from human plasma[14] and aqueous solutions.[41] They observed 150 μg Fe^{3+}/g adsorption capacity from human plasma with selectivity coefficients of 42.6 and 36.1 with respect to Cd^{2+} and Ni^{2+}, respectively. Fe^{3+} adsorption capacity from aqueous solutions was higher (0.76 mg/g) and the selectivity coefficients were 59.7 and 37 for the same ions. In this study, PHEMA-MIP cryogels were adsorbed Fe^{3+} ions with high selectivity by combining the advantage of using supermacroporous adsorbents when the adsorption media is a blood plasma.

Selectivity experiments

The specificity of PHEMA-MIP composite cryogels for Fe^{3+} in the presence of Fe^{2+}, Zn^{2+}, and Ni^{2+} ions was determined by a competitive adsorption experiment that was carried out in human plasma under equilibrium conditions as described in experimental section. Figure 4 shows the adsorption of Fe^{3+}, Fe^{2+}, Zn^{2+}, and Ni^{2+} ions onto the Fe^{3+} imprinted and non-imprinted composite adsorbent. As can be seen from the Figure 4, highest amount of Fe^{3+} adsorption is observed with PHEMA-MIP composite adsorbents. Table II outlines K_d, k, and k' values for Fe^{2+}, Zn^{2+}, and Ni^{2+} with respect to Fe^{3+}, in which case PHEMA-NIP composite cryogel was used as a control adsorbent in parallel. As seen in Table II, The K_d value for PHEMA-NIP (1.84) is observed to be negligible compared to that of PHEMA-MIP (181.30). There also appears that the K_d values determined for Fe^{3+} are significantly higher than those obtained for Fe^{2+}, Zn^{2+}, and Ni^{2+} for the PHEMA-MIP composite cryogel. The selectivity coefficient (k) is inversely related to a competitive affinity binding of a cation (molecule) competing with the template cation (molecule) for the same binding site on the adsorbent. It is observed that k value for the Fe^{3+}-Ni^{2+} cation-couples (k) is 53.48, for Fe^{3+}-Zn^{2+} cation-couple is 49.25 and Fe^{3+}-Fe^{2+}

TABLE II
K_d, k, and k', Values of Ni^{2+}, Zn^{2+}, and Fe^{2+} with Respect to Fe^{3+}

	NIP		MIP		
Metal Ion	K_d	k	K_d	k	k'
Fe^{3+}	1.84	–	181.30	–	–
Ni^{2+}	4.65	0.39	3.40	53.48	135.01
Zn^{2+}	2.30	0.80	3.68	49.25	61.41
Fe^{2+}	2.08	0.94	2.58	53.95	57.54

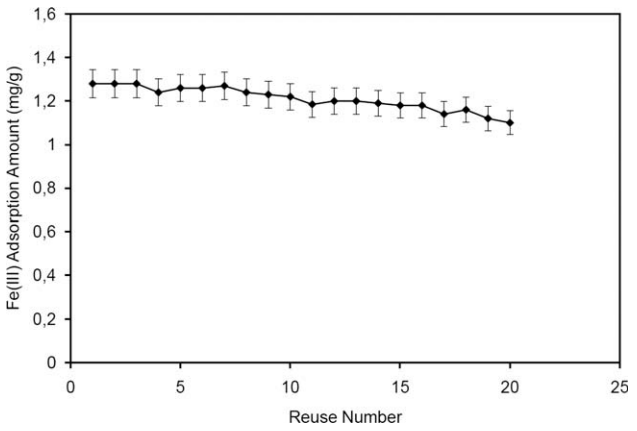

Figure 5 Reusability of PHEMA-MIP composite cryogel.

cation couple is 53.95. Results showed that the ion cavities formed in the MIP beads recognized preferentially Fe^{3+}, indicating that ion cavities matched the size of Fe^{3+} better than Fe^{2+}, Zn^{2+}, and Ni^{2+}. The formation of a coordination complex between the thiol group in the MAC structure and Fe^{3+} is also considered to be another binding mode. The ratio of the *k* values, the relative selectivity coefficients (*k*′), obtained with PHEMA-MIP and PHEMA-NIP composite cryogels were found to be 135.01, 61.41, and 57.54 (Table II) in the presence of the Fe^{3+}-Ni^{2+}, Fe^{3+}-Zn^{2+}, and the Fe^{3+}-Fe^{2+} cation-couples, respectively.

Desorption and reusability

The regeneration of an ion-imprinted adsorbent is crucial in terms of increasing its industrial efficiency at low costs.[42] Thus, bound Fe^{3+} was desorbed out of the pores of PHEMA-MIP composite cryogel by circulating a desorbing agent, a solution of 0.05 M EDTA, through the composite system. There are various known factors that are thought to determine desorption rate of the bound Fe^{3+}, such as an extended hydration of metal cations, the polymer microstructure and the binding strength of metal cations. In this study, desorption ratios were repeatedly determined to be as high 84%. Even though the adsorption–desorption cycles for the PHEMA-MIP composite column were repeated 20 times. For sterilization, after one adsorption–elution cycle, the composite cryogel was washed with 50 m*M* NaOH solution for 30 min. After this procedure, the adsorbent was washed with distilled water for 30 min. As can be seen from the Figure 5 at the end of twenty adsorption–elution cycle, there was no remarkable decrease in the Fe^{3+} ion adsorption capacity. It is therefore quite likely that the PHEMA-MIP composite cryogel can be used many times without decreasing their adsorption capacities significantly.

CONCLUSION

In this study, PHEMA-MIP composite cryogels were prepared and applied to the selective removal of Fe^{3+} ions from β-thalassemia human plasma samples. The results presented here demonstrate that the PHEMA cryogels embedded with MIP beads can be used for the specific removal of Fe^{3+} ions from β-thalassemic human plasma. The adsorption difference between the NIP and MIP bead embedded PHEMA composite cryogels is most probably due to geometric shape affinity (or memory) of Fe^{3+} ions towards the cavities in the Fe^{3+} imprinted poly(GMA-MAC) structure. The relative selectivity coefficient is an indicator to express an adsorption affinity of recognition sites to the imprinted Fe^{3+} ions. Finally, the PHEMA-MIP composite cryogels can be used many times without decreasing their adsorption capacities significantly. The results demonstrate that the MIP based cryogel adsorbents are promising tools for the efficient and specific removal of the excess iron ions from iron overloaded plasma.

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