Binding Behavior of Fe^{3+} Ions on Ion-Imprinted Polymeric Beads for Analytical Applications

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ABSTRACT: We used a molecular imprinting approach to achieve specific metal binding utilizing *N*-methacryloyl-(L)-cysteine methyl ester (MAC) as a metal-complexing ligand. MAC was synthesized using methacryloyl chloride and cysteine methyl ester. Then, Fe^{3+} was complexed with MAC monomer. Fe^{3+}-imprinted poly(hydroxyethyl methacrylate-*N*-methacryloyl-(L)-cysteine methyl ester) [poly(HEMA-MAC)] beads with average size of 63–140 μm were produced by suspension polymerization. After that, the template ions (i.e. Fe^{3+} ions) were removed by 0.1*M* HCl. Fe^{3+}-imprinted beads were characterized by swelling studies, FTIR, and elemental analysis. The Fe^{3+}-imprinted beads with a swelling ratio of 72%, and containing 3.9 mmol MAC/g were used in the binding of Fe^{3+} ions from aqueous solutions, tap water, certified reference serum sample, and real serum sample. Maximum binding capacity, optimum pH, and equilibrium binding time were 107 μmol/g, pH 3.0, and 30 min, respectively. It was observed that even in the presence of other ions, Fe^{3+}-imprinted beads selectively bound Fe^{3+} ions with 97% efficiency. Removal of Fe^{3+} ions from certified reference serum sample was approximately found to be 33%. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 3520–3528, 2006

Key words: molecular imprinting; ion-imprinting polymer; Fe^{3+} removal

INTRODUCTION

Iron is used primarily in the production of steel. Iron and iron compounds are also used in the production of magnets, pigments, abrasives, and polishing compounds. Iron is released to the environment through recycling, waste incineration, and through the use of municipal sewage-sludge that contain iron. Nonoccupational exposure to iron occurs from contact with soil and from ingestion of plant and animal tissue.

Iron is an essential trace element for almost all organisms.[1] The toxic effects of iron overload are well known, especially since the human body has no physiological route for the elimination of excess iron.[2] The toxicity of iron is related to its ability to induce oxidative stress in cells.[3] In an occupational setting, inhalation exposure to iron oxide may cause siderosis. In the nonoccupational population, ingestion of large quantities of iron salts may cause nausea, vomiting, and intestinal bleeding. There is accumulating evidence suggesting that an increase in iron storage may be associated with an increasing risk of developing cancer.[4] Studies have demonstrated that there is an increased risk for developing colorectal carcinoma following ingestion of high amounts of iron.[5] There is also an increase in hepatocellular carcinoma in patients with hereditary hemochromatosis, an inherited disorder in which there is hyperabsorption of iron from the intestinal tract and in lung cancer from exposure to asbestos fibers, which contains ~30% iron by weight.[6]

It has been a long term dream of researchers to build such structures *de novo*, creating tailor-made receptors that are capable of recognizing and binding the desired molecular target with a high affinity and selectivity.[7] Such synthetic materials should be easier to produce and process, less costly, and more stable than biomacromolecules. Moreover, they should be accessible to target molecules for which natural receptors do not exist are difficult to obtain. One surprisingly simple way of generating artificial macromolecular receptors is through the molecular imprinting of polymers.[8–11] Molecular imprinting is a technology to create recognition sites in a macromolecular matrix using a molecular template.[12] In other words, both the shape image of the target and alignment of the functional moieties to interact with those in the target are memorized in the macromolecular matrix for the recognition or separation of the target during formation of the polymeric materials themselves.[13] Molecularly imprinted polymers (MIPs) are easy to prepare, stable, inexpensive, and capable of molecular recognition. Therefore, MIPs can be considered as artificial affinity media. Molecular recogition-based separation tech-

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niques have received much attention in various fields because of their high selectivity for target molecules. Three steps are involved in ion-imprinting process: (i) complexation of template (i.e. metal ions) to a polymerizable ligand, (ii) polymerization of this complex, and (iii) removal of template after polymerization. In the ion-imprinting process, the selectivity of a polymeric adsorbent is based on the specificity of the ligand, on the coordination geometry and coordination number of the ions, and on their charges and sizes.[14–16] Numerous studies describing such methodology were carried out to adsorb metal ions.[17–19]

In this study, the Fe^{3+}-imprinted polymer beads were prepared and they were used for the selective binding of Fe^{3+} ions from complex matrices. *N*-methacryloly-(L)-cysteine methyl-ester (MAC) was synthesized as the metal complexing monomer, with the goal of preparing a solid-phase that has the high selectivity for Fe^{3+}. Usually, MIPs are prepared by the bulk polymerization. The disadvantage of this method is that the obtained block polymers should be crushed, ground, and sieved to produce packing materials.[20] Fe^{3+}-imprinted beads were produce by a suspension polymerization. PHEMA was selected as the base matrix, which has hydrophilic character, minimal nonspecific interactions, high chemical and mechanical stability, and resistance toward microbial and enzymatic attacks.[21–23] After removal of Fe^{3+} ions, Fe^{3+}-imprinted beads were used for the binding of iron from complex matrices. Fe^{3+} binding from complex matrices containing different amounts of metal ions, and selectivity studies of iron versus other interfering metal ions mixture, Fe^{2+}, Cr^{3+}, Cu^{2+}, and Zn^{2+}, were reported here. Finally, use of the Fe^{3+}-imprinted beads in tap water and certified reference serum sample was also discussed.

EXPERIMENTAL

Materials

Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor, and stored at 4°C until use. Benzoyl peroxide (BPO) was obtained from Fluka (Switzerland). Poly(vinyl alcohol) (PVAL; MW, 100,000; 98% hydrolyzed) was supplied from Aldrich Chem (USA). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in a dust-free environment. Stock solutions of 1000 mg/L Fe^{3+} and Fe^{2+} were prepared by dissolving iron nitrate ($Fe(NO_3)3.9H_2O$) (Merck, Darmstadt, Germany) and ferrous chloride ($FeCl_2.4H_2O$) (BDH, Poole, England), respectively. Standard iron solutions were prepared daily by dilution of the stock solutions. All water used in the binding 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*-methacryloly-(L)-cysteine methyl ester

Details of the preparation and characterization of the *N*-methacryloly-(L)-cysteine methyl ester (MAC) was reported elsewhere.[24,25] Briefly, the following experimental procedure was applied for the synthesis of MAC monomer: 5.0 g of cysteine metyl ester and 0.2 g of $NaNO_2$ were dissolved in 30 mL of K_2CO_3 aqueous solution (5%, v/v). This solution was cooled to 0°C. Methacryloyl chloride (4.0 mL) was poured slowly into this solution under nitrogen atmosphere and then this solution was stirred magnetically at room temperature for 2 h. At the end of this period, the pH of this solution was adjusted to 7.0 and then was extracted with ethylacetate. The aqueous phase was evaporated in a rotary evaporator. The residue (i.e. MAC) was crystallized in ethanol and ethylacetate.

Preparation of Fe^{3+}-MAC complex

To prepare MAC–Fe^{3+} complex, solid *N*-methacryloly-(L)-cysteinemethylester (MAC) (0.378 g, 2.0 mmol) was added slowly into 15 mL of ethanol–water mixture (50/50 v/v) and then treated with iron nitrate ($Fe(NO_3)_3.9H_2O$) (0.404 g, 1.0 mmol) at room temperature with continuous stirring for 3 h. Then, the formed metal–monomer complex was filtered, washed with 99% ethanol (250 mL), and dried in a vacuum oven.

Preparation of Fe^{3+}-imprinted poly(HEMA-MAC) beads

Suspension polymerization was used for the preparation of Fe^{3+}-imprinted poly(HEMA-MAC) beads. A typical preparation procedure is given below. Continuous medium was prepared by dissolving poly(vinyl alcohol) (200 mg) in the deionized water (50 mL). For the preparation of dispersed phase, HEMA (4.0 mL), Fe^{3+}–MAC complex (500 mg), EGDMA (8.0 mL), and toluene (12 mL) were mixed and BPO (100 mg) was dissolved in the homogeneous organic phase. The organic phase was dispersed in the aqueous medium by stirring the mixture magnetically (600 rpm), in a sealed pyrex polymerization reactor (volume: 250 mL). The reactor content was heated to polymeriza-

tion temperature (i.e. 65°C) and the polymerization was carried out for 4 h with a 600 rpm stirring rate at 65°C. Then, temperature was increased to 90°C and the polymerization was conducted for 2 h. Final beads were extensively washed with ethanol and water to remove any unreacted monomer or diluent and then stored in distilled water at 4°C. Nonimprinted poly-(HEMA-MAC) beads were prepared in the same way without addition of MAC–Fe^{3+} complex.

After the cleaning procedure, the template (i.e., Fe^{3+} ions) was removed from the polymer beads using 0.1*M* HCl. The imprinted beads were added into the 0.1*M* HCl solution for 48 h at room temperature. This procedure was repeated several times until the template molecule (i.e. Fe^{3+} ions) could not be detected in the filtrate with a graphite furnace atomic absorption spectrophotometer. The template-free polymers were cleaned with 0.1*M* HNO_3 in a magnetic stirrer for 3 h. Then, the beads were filled in a fixed-bed column, and washing solutions (i.e. a dilute HCl solution and a water–ethanol mixture) were recirculated through the system, which also includes an activated carbon column, until the beads are clean. Purity of the beads was followed by observing the change of optical densities of the samples (wavelength: 220–280 nm) taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the beads obtained by using a differential scanning calorimeter microcalorimeter (Mettler, Switzerland). Heating rate was 10°C/min. Optical density of the uncleaned beads was 1.46. But after cleaning operation, this value was reduced to 0.04. In addition, when the thermogram of uncleaned beads was recorded, it has a peak around 60°C. This peak might originate from BPO (it gives radicals at 60°C). After application of this cleaning procedure, no peak between 30 and 100°C was observed on this thermogram. When not in use, the resulting beads were kept under refrigeration in 0.02% NaN_3 solution for preventing of microbial contamination.

Characterization of beads

The average size and size distribution of the beads were determined by screen analysis performed using Standard Test Sieves (Retsch GmbH, Germany). The specific surface area of the beads was determined in BET apparatus. Water uptake ratios of the beads were determined in distilled water. The experiment was conducted as follows: initially, dry beads were carefully weighed before being placed in a 50 mL vial containing distilled water. The vial was put into an isothermal water bath at a fixed temperature (25°C) for 2 h. The bead sample was taken out from the water, wiped using a filter paper, and weighed. The weight ratio of dry and wet samples was recorded. The water content of the beads was calculated using the weights of beads before and after uptake of water. The beads were examined using scanning electron microscopy (SEM). The samples were initially dried in air at 25°C for seven days before being analyzed. A fragment of the dried bead was mounted on a SEM sample mount and was sputter coated for 2 min. The sample was then mounted in a scanning electron microscope (Model: JSM 5600, Jeol, Japan). The surface of the sample was then scanned at the desired magnification to study the morphology of the beads. To evaluate the degree of MAC incorporation, the beads were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932). FTIR spectra of MAC and the imprinted beads were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The beads (about 0.1 g) were thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany) and pressed into a pellet, and the FTIR spectrum was then recorded.

Fe^{3+} ions binding/desorption studies

Binding of Fe^{3+} and Fe^{2+} ions on the Fe^{3+}-imprinted poly(HEMA-MAC) beads were studied in batch experiments. Effects of Fe^{3+} and Fe^{2+} concentrations and pH of the medium on the binding rate and binding capacity were studied. Aliquots of aqueous solutions (25.0 mL) containing different amount of Fe^{3+} and Fe^{2+} ions in the range of 1.0–70.0 mg/L were treated with the polymer beads at different pH in the range of 2.0–5.0 (adjusted with HCl-NaOH). Polymer beads (50 mg) were stirred with a iron(III) nitrate and iron(II) chlorine salt solutions at room temperature for 1 h with stirring rate 400 rpm. The concentrations of the Fe^{3+} ions in the aqueous phase after the desired treatment periods was measured using a Perkin–Elmer Analyst 800 atomic absorption spectrometer with deuterium background correction was used. A Perkin–Elmer hollow cathode iron lamp was used. The working current/wavelength was 30 mA 248.3 nm with spectral bandwith of 0.5 nm. The instrument response was periodically checked with known Fe^{3+} and Fe^{2+} standard solutions. The experiments were performed in replicates of three as well. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 96% were calculated for each set of samples to determine the margin of error. The amount of iron binding per unit mass of the beads was evaluated by using the mass balance.

Selectivity experiments

To show Fe^{3+} specificity of the Fe^{3+}-imprinted beads, Fe^{2+} was chosen its different oxidation state and its toxic behavior in this state. Zn^{2+} and Cu^{2+} were cho-

sen, which have toxic behavior, and Cr^{3+} was chosen, which has the same oxidation state as Fe^{3+}.

In selectivity experiments, competitive binding of Fe^{3+}/Fe^{2+}, Fe^{3+}/Cr^{3+}, Fe^{3+}/Cu^{2+}, and Fe^{3+}/Zn^{2+} from their binary solutions were studied. Solutions containing 25 mg/L of metal ions were treated with 50 mg of Fe^{3+}-imprinted beads for 30 min at pH 3.0, in the flasks stirred magnetically at 400 rpm. After binding equilibrium, the concentration of the metal ions in the remaining solution was measured by a FAAS system. A Shimadzu M 1240 UV–vis spectrophotometer was used for the determination of Fe^{2+} in the mixture of Fe^{3+}/Fe^{2+}. Spectrophotometric detection was performed at a wavelength of 510 nm.

The distribution coefficient (K_d) gives the ratio of the amount of metal ion bound by 1.0 g of the adsorbent to the amount of metal ion remained in 1 mL of the solution and the distribution ratio (k) indicates the strength of metal-binding by adsorbent.

Distribution and selectivity coefficients of Fe^{2+}, Cr^{3+}, Cu^{2+}, and Zn^{2+} with respect to Fe^{3+} were calculated as explained by the following equation.

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

Where K_d is the distribution coefficient, C_i and C_f represent the initial and final solution concentrations, respectively. V is the volume of solution used for the binding and m is the weight of polymer used. The selectivity coefficient k for the binding of a specific metal ion in the presence of competitor species can be obtained from equilibrium binding data according to

$$k = K_{\text{template metal}}/K_{\text{interterent metal}} \quad (2)$$

Effect of interfering ions

The effect of interfering ions has also been studied. Sample solutions containing 5.0 mg/L of Fe^{3+} ions and various amounts of Na+, Mg^{+2}, and Ca^{+2} ions as interferants were examined by the general procedure. The chloride, nitrate, and carbonate ions are found to be present in natural water and have the capability to complex with many metal ions. Consequently, they may reduce the binding amount of metal ions. Thus, the effect of NaCl, KCl, $Mg(CO_3)_2$, and $Ca(NO_3)_2$ on the binding of Fe^{3+}-imprinted beads was studied under the optimum conditions.

Desorption and reuse

Desorption of the bound Fe^{3+} ions from the Fe^{3+}-imprinted beads was also studied in batch experimental set-up. HCl, HNO_3, EDTA and NH_4SCN were compared for desorption of Fe^{3+} ions from the Fe^{3+}-imprinted beads after the binding step. In this study, equilibrium desorption time was found to be 30 min at a stirring rate of 400 rpm at room temperature. The final Fe^{3+} ions concentration in the desorption medium was measured by AAS. The desorption ratio was calculated from the amount of Fe^{3+} ions adsorbed on the beads and the final Fe^{3+} ions concentration in the desorption medium.

To obtain the reusability of the Fe^{3+}-imprinted beads, binding-desorption cycle was repeated five times by using the same imprinted beads.

Removal of Fe^{3+} ions from tap-water and certified reference serum

To estimate the accuracy of the procedure, different amounts of the Fe^{3+} ions were spiked in tap water. Sample solution (20 mL) was treated under the general binding-desorption procedure. Removal of Fe^{3+} ions from certified reference serum sample with using Fe^{3+}-imprinted beads was also investigated. This reference material is produced from serum collected from thoroughly controlled voluntary blood donors. To determine the removal of Fe^{3+} ions from the serum, Fe^{3+}-imprinted beads were used for serum samples containing different amounts of Fe^{3+} ions.

RESULTS AND DISCUSSION

Properties of polymer beads

In the suspension polymerization, the yield of spherical beads with a size range of 63–140 μm in diameter was found to be 96.8% (w/w) based on the monomers initially charged in the polymerization reactor. The specific surface areas were found to be 18.9 m^2/g for nonimprinted and 46.5 m^2/g for imprinted polymers. The equilibrium swelling ratios of the nonimprinted and imprinted beads are 72 and 78%, respectively. Compared with poly(HEMA-MAC), the water uptake ratio of the Fe^{3+}-imprinted beads increased. Removal of the template yielded a functional polymer matrix with cavities and spatial arrangements of functional groups. These cavities in the polymer structure introduced more hydrodynamic volume into the polymer chains, which can result uptake in the more water molecules by polymer matrix. The porous character of the Fe^{3+}-imprinted poly(HEMA-MAC) beads are exemplified by the scanning electron micrographs in Figure 1.

N-methacryloly-(L)-cysteine methyl ester (MAC) was selected as the comonomer and ion-imprinted monomer for the selective separation of Fe^{3+} ions. In the first step, MAC was synthesized from cysteine methyl ester and methacryloyl chloride and complexed with Fe^{3+} ions. The MAC–Fe^{3+} complex is shown in Figure 2. The incorporation of the MAC was found to be 3.9 mmol/g polymer by using nitrogen

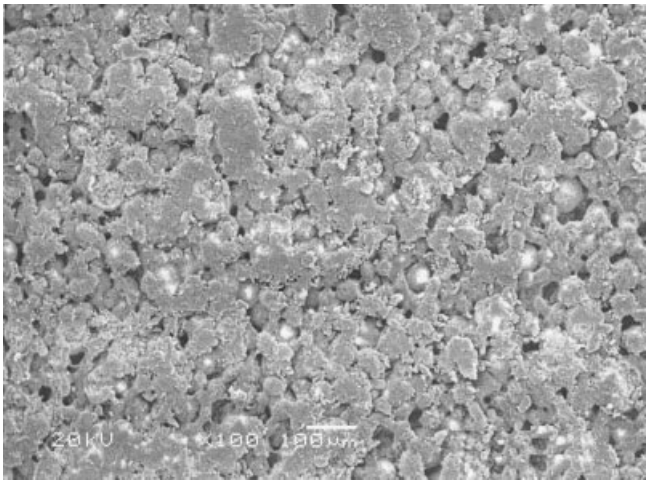

Figure 1 SEM Photograph of Fe^{3+}-imprinted poly(HEMA-MAC) beads.

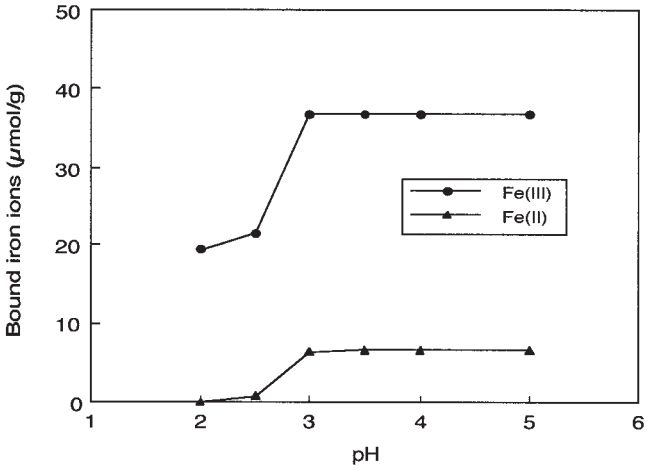

Figure 3 Effect of pH on Fe^{3+} and Fe^{2+} binding. Fe^{3+} and Fe^{2+} concentration, 5.0 mg/L; binding time, 30 min; *T*, 25°C.

stoichiometry. Note that HEMA and other polymerization ingredients do not contain nitrogen. This nitrogen amount determined by elemental analysis comes only from incorporated MAC groups into the polymeric structure.

FTIR spectrum of MAC has the characteristic stretching vibration amide I and amide II absorption bands at 1651 and 1558 cm^{-1}, and carbonyl band at 1724 cm^{-1}. For the characteristic determination of complex, due to linear coordinate covalent complex formation, the characteristic strong S—H stretching vibration bands at 1130 and 970 cm^{-1} slips to the higher frequency field at 950 and 750 cm^{-1}, as a result of decreasing the electron density of sulfhydryl group of MAC monomer. The Fe—S bands occur at 360 and 348 cm^{-1} but these bands can not be observed because the instrumental region is up to 400 cm^{-1}. The FTIR spectrum of Fe^{3+}-imprinted poly(HEMA-MAC) beads have the characteristic stretching vibration band of hydrogen bonded alcohol, O—H around 3649 cm^{-1}, amide I and amide II absorption bands at 1645 and 1456 cm^{-1}, respectively.

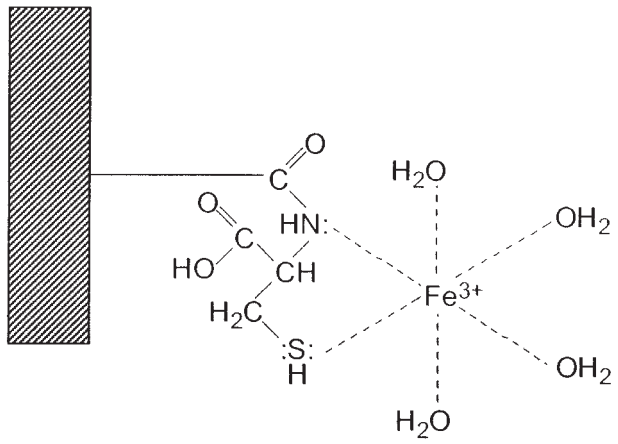

Figure 2 The MAC- Fe^{3+} complex monomer.

Binding of Fe^{3+} and Fe^{2+} ions from aqueous solutions

Effect of pH

The metal ion complexation of polymeric ligands and speciation are highly dependent on the equilibrium pH of the medium. In the present study, we changed the pH range between 2.0 and 5.0. The effect of pH on the Fe^{3+} and Fe^{2+} binding of the Fe^{3+}-imprinted beads was shown in Figure 3. As seen here, binding of iron ions increased with increasing pH and then reached almost a plateau value around pH 3.0. The increasing pH of the solution favors complex formation between the sulfydryl groups of MAC in the ion cavities and iron ions. The specific adsorption of Fe^{3+} ions via the MAC groups, which was pH dependent, was much higher (up to 36.8 μmol/g) than Fe^{2+} ions (6.5 μmol/g). Iron binding around pH 2.0–2.5 was low, maybe due to protonation of the functional groups on the MAC structure. High binding at increasing pH values shows that Fe^{3+} ions interact with MAC groups by chelating and cation-exchange.

Equilibrium binding time

Figure 4 shows the time dependence of the binding capacities of Fe^{3+} and Fe^{2+} ions on Fe^{3+} imprinted beads. Note that these batch experiments were performed by using single (not together) solutions of the Fe^{3+} and Fe^{2+} ions. As seen here, iron binding increases with the time during the first 30 min and then levels off as equilibrium is reached. This fast binding equilibrium is most probably due to high complexation and geometric shape affinity (or molecular rec-

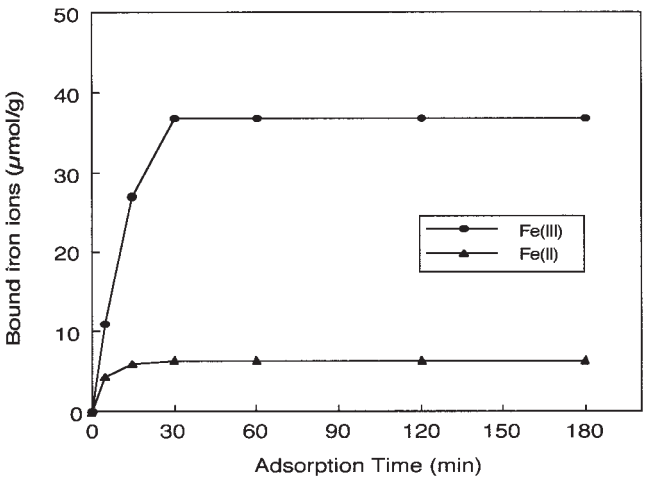

Figure 4 Time dependent binding of Fe^{3+} and Fe^{2+} ions on the Fe^{3+}-imprinted beads; metal ion concentration, 5.0 mg/L; pH, 3.0; *T*, 25°C.

ognition) between Fe^{3+} ions and Fe^{3+} cavities in the imprinted beads structure. It is well known that removal of the template from the polymeric matrix leaves cavities of complementary size, shape, and chemical functionality to the template.

Binding capacity

Fe^{3+} and Fe^{2+} ions binding capacities of the Fe^{3+}-imprinted beads are presented as a function of the initial concentration of metal ions within the aqueous binding medium in Figure 5. These batch experiments were performed by using single solutions of the interested ions. Fe^{3+} binding capacity of the Fe^{3+}-imprinted beads increased first with increasing concentration of Fe^{3+}, then reached a plateau value at about

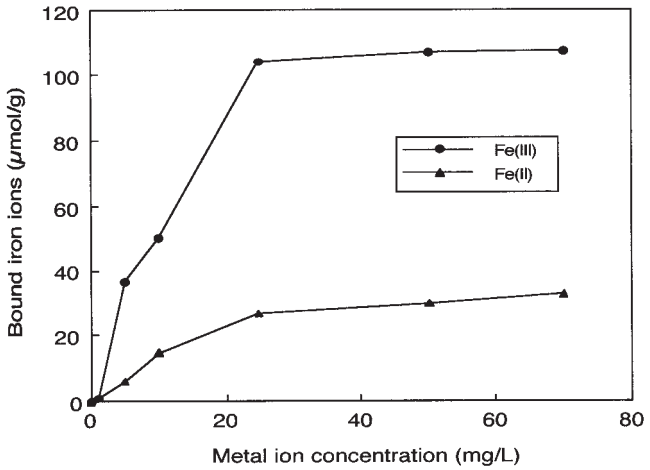

Figure 5 Binding capacity of Fe^{3+} and Fe^{2+} ion concentration on binding of metal ions on the Fe^{3+}-imprinted poly-(HEMA-MAC) beads; pH, 3.0; *T*, 25°C.

TABLE I
The Effect of Imprinting on Selectivity

Metal ions	Bound metal ions[a] (mg/g)	K_d (mL/g)	k
Fe^{3+}	5.8[b]	552.4	—
Fe^{2+}[c]	1.1	50.5	10.9
Cr^{3+}	1.2	54.5	10.1
Cu^{2+}	—	—	—
Zn^{2+}	—	—	—

[a] Each experiment was repeated three times.
[b] The same value was obtained all the experiments.
[c] Spectrophotometric 1,10-Phenanthroline method was used for the determination of Fe^{2+} concentration in Fe^{3+}/Fe^{2+} mixture.

an initial Fe^{3+} concentration of 25 mg/L. The maximum binding capacities of Fe^{3+}-imprinted beads are 107.1 and 32.9 μmol per gram of the beads for Fe^{3+} and Fe^{2+}, respectively. It should be noted that Fe^{3+}-imprinted beads have a significantly higher selectivity for Fe^{3+} than Fe^{2+}.

Competitive binding

To obtain the selectivity of Fe^{3+}-imprinted beads, competitive binding of Fe^{3+}/ Fe^{2+}, Fe^{3+}/Cr^{3+}, Fe^{3+}/Cu^{2+}, and Fe^{3+}/Zn^{2+} from their binary solutions was investigated. Solutions containing 25 mg/L of metal ions were treated with 50 mg of Fe^{3+}-imprinted beads for 30 min at pH 3.0. The distribution coefficient (K_d) gives the ratio of the amount of metal ion adsorbed by 1.0 g of the beads to the amount of metal ion remained in 1.0 mL of the solution and distribution ratio (k) indicates the strength of metal-binding by adsorbent. Binding capacities, K_d and k values are given in Table I.

It should be noted that the adsorbed value of Fe^{3+} ions in single solution (corresponding a 25 mg/L Fe^{3+} initial concentration) is 6.01 mg/g. The equilibrium binding capacity of Fe^{3+} ion was reduced by 3% in the presence of an equal concentration of other metal ions; in the other words, these metal ions sligtly inhibit the complexation between Fe^{3+} ions and template groups in the beads structure. The K_d value of Fe^{3+} is very high compared to those of other metal ions. Although Cr^{3+} ion has same oxidation state as Fe^{3+}, the Fe^{3+}-imprinted beads showed excellent selectivity for the Fe^{3+} ions. This means that Fe^{3+} can be determined even in the presence of Fe^{2+}, Cr^{3+}, Cu^{3+}, and Zn^{2+} interferences. This shows the chemical nature of the template groups in the imprinted beads is of great importance for the binding process.

Equilibrium studies

The most widely used isotherm equation for modeling equilibrium data is Langmuir equation, which for dilute solutions may be represented as

TABLE II
Values of Calculated Constants in the Langmuir and Freundlich Models

Model	Values
Langmuir	
a_L/K_L	6.16
K_L	0.1
$R2$	0.9979
Freundlich	
a	0.2
b	0.409
R^2	0.9883

$$q_e = K_L C_e[1/(1 + a_L C_e)] \quad (3)$$

Where q is the amount of adsorbed metal ions in the adsorbent, C_e is the equilibrium metal ion concentration in solution. The constants K_L and a_L are the characteristics of the Langmuir equation and can be determined from a linerized form of the above equation:

$$C_e/q_e = (1/K_L) + (a_L/K_L)C_e \quad (4)$$

Therefore, a plot of C_e/q_e versus C_e gives a straight line of slope a_L/K_L. The constant K_L is the Langmuir equilibrium constant and the ratio a_L/K_L gives the theoretical monolayer saturation capacity. The Langmuir equation is applicable to homogeneous binding where each metal ion-imprinted bead binding process has equal binding activation energy.

The Freundlich expression is an empirical equation based on sorption on a heterogenous surface. The Freundlich equation is commonly presented as

$$q_e = aC_e^b \quad (5)$$

and the equation may be linerized by taking logarithms

$$\ln q_e = b \ln C_e + \ln a \quad (6)$$

therefore, a plot of ln q_e versus ln C_e enables the constant a and exponent b determined.

The parameter a is equivalent to q_{max} in the Langmuir isotherm. The Freundlich isotherm does not predict saturation of the solid surface by the adsorbate, thus infinite surface coverage is predicted mathematically.

The Langmuir and Freundlich constants along with the correlation coefficients (R^2) have been calculated from the corresponding plots for Fe^{3+} ions on the binding of Fe^{3+}-imprinted beads and the results are presented in Table II.

The correlation regression coefficients show that the binding process can be well defined by the Langmuir equation. The Langmuir fit is considered to be evidence that binding stops at monolayer, consistent with specific and strong binding onto specific sites. Since the exchange reaction between surface sites previously adsorbed ions is of only a monolayer or less, there is an accumulation of matter at the solid-solution interface without the creation of a three-dimensional (3D) structure.

Desorption and reuse

To be useful in removal processes, adsorbed species should be easily desorbed under suitable conditions and adsorbents should be used many times to decrease material costs. Main advantages of the ion imprinted beads used as an adsorbent are the regeneration and recycling properties of this matrix. Desorption of the bound Fe^{3+} ions from the Fe^{3+}-imprinted beads was also studied in a batch experimental setup. HCl, HNO_3, EDTA, and NH_4SCN were compared for desorption of Fe^{3+} ions from the imprinted beads after the binding step. The desorption ratios of Fe^{3+} ions were not quantitative with NH_4SCN and EDTA solutions. It was seen that Fe^{3+} ions could be effectively desorbed with 20 mL of 0.1*M* HCl and 0.1*M* HNO_3 and desorption ratios greater than 96.2% and 95.5%, respectively (Table III). This means that HCl and HNO_3 breaks down the interaction forces between Fe^{3+} ions and binding sites in the cavities of the ion-imprinted beads.

To obtain the reusability of the Fe^{3+}-imprinted beads, binding-desorption cycle was repeated five times by using the same imprinted beads. The results showed that Fe^{3+}-imprinted beads could be repeatedly used in Fe^{3+} binding without any detectable loss in the initial binding capacity (Fig. 6). Removal of Fe^{3+} ions from tap-water and certified reference serum

To estimate the accuracy of the procedure, different amounts of the Fe^{3+} ions were spiked in tap water. Sample (20 mL) solution was treated under the general binding-desorption procedure. The results reported in Table IV. A good agreement was obtained between the added and measured analyte amounts.

Removal of Fe^{3+} ions from certified reference serum sample (SERO 201405-Seronorm Trace Elements Serum, Level 1) and real serum sample with using Fe^{3+}-imprinted beads was also investigated. This reference

TABLE III
Desorption Ratios of Fe^{3+} Ions from the Imprinted Beads

Desorption reagent (0.1*M*)	% Desorption
HCl	96.2
HNO_3	95.5
EDTA	63.4
NH_4SCN	56.7

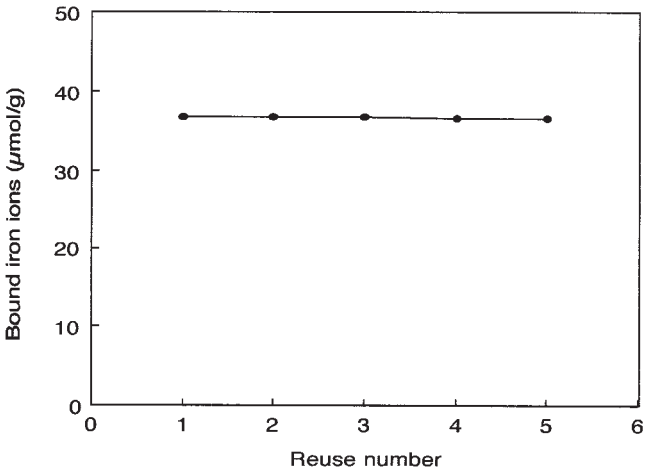

Figure 6 Binding/desorption cycle of Fe^{3+}-imprinted beads. Desorption agent, 0.1*M* HCl. Fe^{3+} concentration, 5.0 mg/L.

material is produced from serum collected from thoroughly controlled voluntary blood donors. Each unit separately controlled and found negative for HBS antigen and HIV I, II, and hepatitis C antibodies. No preservatives are added to the samples and contain all normal constituents, which ensure the control and test samples to be analyzed under the same conditions.

To investigate the removal of Fe^{3+} ions from the serum, Fe^{3+}-imprinted beads were used for serum samples containing different amounts of Fe^{3+} ions. For unpoisoned serum (iron content in this case was 1.25 mg/L), different amount of $Fe(NO_3)_3.9H_2O$ were added (1.25, 4.00 mg/L) and serum samples was treated with Fe^{3+}-imprinted beads.

The removal results of Fe^{3+} ions from certified reference serum sample and real serum sample were shown in Table V. Fe^{3+}-imprinted beads have shown relatively close performance during the treatment with certified reference serum sample and real serum sample. It is known that the iron in plasma is tightly bound to its transport protein transferrin. Normal serum iron levels fluctuate around 1.0 mg/L, and typically only 30% iron binding sites of transferring are occupied, making the total iron binding capacity 3.0 mg/L. From the results shown in Table V, it can be seen that for unpoisoned serum (iron content in this case was 1.25 mg/L) only a small amount of iron was removed, while more iron was removed at higher iron

TABLE IV
Removal of Fe3+ Ions from Tap Water ($N = 5$)

Iron added (μg/mL)	Iron found (μg/mL)	Recovery (%)
0.00	0.062 ± 0.002	—
2.50	2.41 ± 0.11	96
5.00	4.86 ± 0.17	97

TABLE V
Removal of Fe^{3+} Ions in Certified Reference Serum and Real Serum Sample

	Certified values (mg/L)	Found (mg/L)	Added (mg/L)	Iron removal[a] (%)
SERO 201405	1.25 ± 0.1[b]	—	—	30
	—		1.25	35
		—	4.00	37
Real serum	10.1 ± 1.5[c]	—		34
	—	10.8 ± 1.7[c]	—	32

[a] Mean value of three determinations.
[b] ICP-AES.
[c] FAAS.

concentrations. It is interesting to note that the iron content of the treated serum was 0.875 mg/L, and this value is the region of normal serum iron level.

In addition, the overloaded iron was removed by the Fe^{3+}-imprinting beads. As a result, the binding capacity of Fe^{3+}-imprinting beads was close to binding capacity of beads in noninterfering medium.

CONCLUSIONS

MIPs are materials that can be readily tailored with selectivity and affinity for guest molecules.[8] Accordingly, MIPs have been utilized in many analytical applications that require molecular recognition, including sensors, adsorbents, immuno-type assays, and chromatographic stationar phases.[8–19] Up to date, molecular imprinted polymers have been produced by bulk polymerization. These bulk polymers have to be ground and sieved to obtain particles of the desired dimensions for further use.[26–28] This process is not only wasteful and time consuming but also produces irregularly shaped particles, which are unfavorable for applications, and therefore spherical MIPs are more desirable.[28–34] Molecularly imprinted beads that are spherical in shape were prepared by suspension polymerization. The average size of the beads was controlled to be between 63 and 140 μm in diameter. The binding was relatively fast and the time required to reach equilibrium conditions was about 30 min. The maximum binding capacity for Fe^{3+} ions was 107.1 μmol per gram dry weight of beads. This fast binding equilibrium is most probably due to high complexation and geometric affinity between Fe^{3+} ions and Fe^{3+} cavities in the beads structure. The binding values increased with increasing concentration of Fe^{3+} ions, and a saturation value is achieved at ion concentration of 30 mg/L, which represents saturation of the active binding cavities on the Fe^{3+}-imprinted beads. The relative selectivity coefficient is an indicator to express an binding affinity of recognition sites to the imprinted Fe^{3+} ions. The results showed that the im-

printed beads for Fe^{3+}/Cr^{3+} and Fe^{3+}/Fe^{2+} were 10.1 and 10.9 times greater than nonimprinted matrix, respectively. The desorption time was found to be 30 min. The Fe^{3+}-imprinted beads can be used many times without decreasing their binding capacities significantly.

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