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Ion-Imprinted Thermosensitive Polymers for Fe^{3+} Removal from Human Plasma

Seçil Utku[1], Erkut Yılmaz[1], Deniz Türkmen[1], Lokman Uzun[1], Bora Garipcan[2], Rıdvan Say[3], Adil Denizli[1]*

[1]Hacettepe University, Department of Chemistry, Ankara, Turkey

[2]Hacettepe University, Bioengineering Division, Ankara, Turkey

[3]Anadolu University, Department of Chemistry, Eskişehir, Turkey

Abstract

Thermosensitive gels have attracted a great deal of attention for the applications of drug delivery systems, actuators, separation and removal of biological compounds and metals. Poly(*N*-isopropylacrylamide) (poly(NIPA)), a representative gel, has a lower critical solution temperature (LCST) in the vicinity of 32°C i.e. showing hydrophilicity and hydrophobicity in water at lower and higher temperatures, respectively. Novel adsorbents using thermosensitive gels for trapping metals were reported where poly(NIPA) was used as a thermosensitive backbone polymer. A chelating group, which interacts with heavy metals, was introduced into poly(NIPA) with a molecular imprinting technique using a specific metal as the template. Although it is emphasized that metals play important roles in biological processes and some of them are classified as essential, the toxic symptoms will manifest when a metal ion level exceeds a certain threshold level. Many symptoms of iron toxicity, for example heart attacks, diabetes, arthritis, depression and liver failure, are arised from the absorption of iron in unacceptably high concentrations because of a genetic failure or by accidental ingestion. The aim of this study is to prepare ion-imprinted polymers for the removal of iron from aqueous solutions and solutions thalassemia patient`s plasma. N-methacryloyl-(L)-cysteine (MAC) was selected as the metal complexing monomer, with the goal preparing a solid-phase which has a high selectivity for Fe^{3+} ions. Poly(NIPA) was used as a thermosensitive backbone polymer matrix. A chelating group [N-methacryloyl-(L)-cysteine (MAC)] which interacts with Fe^{3+} ions, was introduced into poly(NIPA) with a molecular imprinting technique. After removal of the Fe^{3+} ions, adsorption of Fe^{3+} ions from thalassemia patient's plasma both on the poly(NIPA-MAC) and Fe^{3+}-imprinted poly(NIPA-MAC) particles were studied in batch-wise.

Key Words: Thermosensitive polymers, poly(NIPA), Ion imprinting, Thalassemia, Affinity binding.

INTRODUCTION

Considerable researches have been focused on polymeric materials those change their structure and functions responding to external physical, chemical, and electrical stimuli (light, temperature, pH, substance concentration, solvent composition, and electric fields, etc.). These materials termed 'intelligent materials', sense one or more external stimuli (sensor) as signals, judge the magnitude of these signals (processor), and change their structure and functions in direct response (effecter). The response of the intelligent materials toward above described stimuli induce several kinds of changes such as phase, shape, surface energies,

* Correspondence to: Adil Denizli,
Hacettepe University, Department of Chemistry, Ankara-Turkey

Tel: +90312 297 7983 Fax: +90312 299 2163
E-mail: denizli@hacettepe.edu.tr

permeation rates, reaction rates, and molecule recognition. Introduction of stimuli-responsive polymeric materials as switching sequences into both artificial materials and bioactive compounds (peptides, proteins, nucleic acids, and others) permit modulation of their structure induced by corresponding external stimuli. 'On–off' switching of their respective functions, thus, can be achieved at molecular level [1-5]. Intelligent materials embodying these concepts might contribute to establish fundamental principles for fabrication of novel systems.

Thermosensitive gels have attracted a great deal of attention for the applications to drug delivery systems, actuators and so on. Poly(*N*-isopropyl acrylamide) (poly(NIPA)), a representative gel, has a lower critical solution temperature (LCST) in the vicinity of 32°C [6,7], i.e. showing hydrophilicity and hydrophobicity in water at lower and higher temperatures, respectively. Novel adsorbents using thermosensitive gels for trapping heavy metals were reported several papers [8-11], where poly(NIPA) was used as a thermosensitive backbone polymer. A chelating group, which interacts with heavy metals, was introduced into poly(NIPA) with a molecular imprinting technique [12] using a specific metal as the template. The molecular imprinted adsorbents reconstruct multi-point adsorption sites at a specific temperature and disrupt them through swelling deformation at a lower temperature. The adsorbents, therefore, are suitable for the temperature swing adsorption (TSA), i.e. the control of adsorption and desorption of a specific heavy metal with the change in temperature. TSA using the adsorbents described here provides an energy-saving and environmentally friendly process for the separation of both undesirable and valuable metals ions in aquatic environments, industrial effluents and biological samples.

Molecular recogition-based separation techniques have received much attention in various fields because of their high selectivity for target molecules [13,14]. Molecular imprinting has been recognized as a promising technique for the preparation of such systems. Three steps are involved in ion-imprinting process: (i) Complexation of metal ion (template) to polymerizable ligand, (ii) Copolymerization of this complex, (iii) Removal of metal ion after copolymerization. After removal of target ion, the prepared matrix is put into a solution containing metal ions from which the imprinted ion should thus be preferentially extracted. In ion-imprinting process, the selectivity of an adsorbent is based on the specificity of the ligand, on the coordination geometry and coordination number of the ions, on their charges and sizes [13,14].

Numerous studies describing such methodology were carried out in order to adsorb metal ions [15-19] but not so many studies concerning metal removal from human plasma using ion-imprinting materials were reported in the literature [20,21].

Thalassemia is the world's most common hereditary disease, and is a paradigm of monogenic genetic diseases [22]. Because of increased population mobility, the disease is found today throughout the world, even in places far from the tropical areas in which it arose. Therapy of thalassemia has in the past been confined to transfusion and chelation [23]. Recently, novel modes of therapy have been developed for thalassemia, based on the pathophysiology and molecular pathology of the disease, both of which have been extensively studied. The therapeutic modalities currently in use for the supportive treatment of thalassemia, both those that are standard therapy and those that are in clinical trials are such as; transfusion, chelation (intravenous and oral), antioxidants and various inducers of fetal hemoglobin (hydroxyurea, erythropoietin, butyrates, hemin) [24]. Most of the newer therapies are suitable primarily for

thalassemia intermedia patients. In addition, the treatment modalities currently in use for the curative treatment of thalassemia major will be discussed, including bone marrow transplantation in its various forms. Experimental therapeutic methods, such as intrauterine bone marrow transplantation and gene therapy, are included. Physicians caring for thalassemia patients have an increasing variety of treatment options available. Future clinical studies will determine the place of newer agents and modalities in improving the quality of life as well as the life expectancy of thalassemia patients [24].

In the present study, we prepared ion-imprinted polymeric particles, which were used for the selective removal of Fe^{3+} ions from thalassemia patient`s plasma. We synthesized N-methacryloyl-(L)-cysteine (MAC) as the metal complexing monomer, with the goal preparing a solid-phase which has a high selectivity for Fe^{3+} ions. Poly(NIPA) was used as a thermosensitive backbone polymer matrix. A chelating group [N-methacryloyl-(L)-cysteine (MAC)] which interacts with Fe^{3+} ions, was introduced into poly(NIPA) with a molecular imprinting technique. After removal of Fe^{3+} ions, Fe^{3+}-imprinted polymeric particles were used for the removal of the Fe^{3+} ions from thalassemia patient's plasma. Selectivity studies of Fe^{3+} versus other interfering metal ions mixture which are Ni^{2+} and Cd^{2+} are reported here. Adsorption of Fe^{3+} ions from thalassemia patient's plasma both on the poly(NIPA-MAC) and Fe^{3+}-imprinted poly(NIPA-MAC) particles were studied in batch-wise.

MATERIALS AND METHODS

Materials

N-isopropylacrylamide (NIPA), the crosslinker *N,N*-methylenebis(acrylamide) (MBAAm), the initiator ammonium persulfate (APS), the accelerator *N,N,N',N'*-tetramethylenediamine (TEMED) were obtained from Aldrich Chem. Co. (USA). NIPA monomer was purified by crystallization from toluene/n-hexane mixture before polymerization. Methacryloylchloride and L-cysteine were obtained from Fluka (Germay). All other chemicals were of reagent grade and were purchased from Merck AG (Darmstadt, Germany). All water used in the adsorption 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. Resulting purified water (deionized water) has a specific conductivity of 18.2 µS.

Preparation of Polymeric Gels

Synthesis of N-methacryloyl-L-cysteine

The following experimental procedure was applied for the synthesis of N-methacryloyl-L-cysteine (MAC) monomer: 5.0 g of L-cysteine 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. 4.0 mL of methacryloyl chloride 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 MAC-Fe^{3+} Complex

In order to prepare, MAC-Fe^{3+} complex, 0.404 g iron nitrate ($Fe(NO_3)_3.9H_2O$) (1.0 mmol) was dissolved in 15 ml H_2O. 0.38 g N-methacryloyl-L-cysteine (MAC) (2.0 mmol) was added slowly to this solution with continuous stirring at room temperature. The solution was allowed to be stirred for 3 h. MAC-Fe^{+3} complex solution was used directly in the polymerization procedure.

Preparation of Fe^{3+}-imprinted poly(NIPA-MAC) Gel

The Fe^{3+} imprinted poly(NIPA-MAC) was prepared by free radical polymerization. *N*-isopropyl acrylamide (NIPA) monomer was purified by crystallization from toluene/n-hexane mixture. *N,N*-methylenebis(acrylamide) (MBAAm) was used as a cross-linker agent. *N,N,N',N'*-tetramethylene diamine (TEMED) and ammonium persulfate (APS) were used as the accelerator and initiator, respectively. The polymerization was performed in H_2O at room temperature for 24 hours in poly(vinylchloride) plastic tubes. The preparation conditions are summarized in Table 1. The formed gel was washed extensively with deionized water and dried in lyophilizator. Poly(NIPA) gels were prepared without chelating monomer and non-imprinted poly(NIPA-MAC) gels were synthesized using only MAC monomer.

Removal of the Template (Fe^{3+} ions)

In order to remove the template (Fe^{3+} ions), from lyophilized poly(NIPA-MAC) Fe^{3+} complex, the polymeric gel was ground to make particles. The particles of poly(NIPA-MAC)Fe^{3+} complex, were added into the 0.1 M HNO_3 acidic solution for 12 h at room temperature. The template free polymers were centrifuged and dried in lyophilizator.

Characterization of Poly(NIPA-MAC) Gels

The swelling kinetic of the poly(NIPA-MAC) gels was measured gravimetrically. The dried samples were placed in distilled water at 22°C and removed from water at regular time intervals. After the water on the surfaces of the gels was wiped off with moistened filter paper, the weights of the gels were recorded. The swelling ratio was defined as follows:

Table 1. Preparation conditions of Fe^{3+} impinted poly(NIPA-MAC) gels.

Monomer	: NIPA	0.150 g
Chelating Monomer	: MAC-Fe^{3+} complex	1 mL
Cross-linker	: MBAAm	0.02 g
Accelerator	: TEMED	100 µL
Initiator	: APS	0.01 g

For the temperature-response studies, the poly(NIPA-MAC) gels were equilibrated in distilled water at temperatures ranging from 5 to 60°C. The poly(NIPA-MAC) gels were allowed to swell in distilled water for at least 24 h at each predetermined temperature, controlled up to ±0.1°C in a constant-temperature water bath (Julabo, F34, Germany). The gravimetric method was employed to study the poly(NIPA-MAC) gel swelling ratio for finding Low Critical Solubility Temperature (LCST). After immersion in distilled water at a predetermined temperature, the gels were removed from the water and blotted with wet filter paper for the removal of excess water on the gel surface; they were then weighed. After this weight measurement, the gels were re-equilibrated in distilled water at another predetermined temperature, and their swollen weight was determined. The average values of three measurements were taken for each gel, and the equilibrium swelling ratio was calculated as follows:

FTIR spectra of MAC and poly(NIPA), and the Fe^{3+}-imprinted and template removed poly(NIPA-MAC) gels were obtained by using a FTIR spectro-photometer (FTIR 8000 Series, Shimadzu, Japan). The dry gels (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a pellet and the FTIR spectrum was then recorded.

The proton NMR spectrum of MAC monomer was taken in $CDCl_3$ on a JEOL GX-400-300 MHz instrument. The residual non-deuterated solvent (TMS) served as an internal reference. Chemical shifts were reported in ppm (δ) downfield relative to TMS.

Blood Compatibility Studies

Coagulation Time (CT)

Poly(NIPA) and Fe^{3+}-imprinted poly(NIPA-MAC) particles were incubated in 0.1 M phosphate buffer solution (pH: 7.4) for 24 h at room temperature and washed on a glass filter with 0.5 M NaCl solution and distilled water. Fresh frozen pooled human plasma (0.1 mL) was preheated to 37°C for 2 min and then 10 mg of non-imprinted and imprinted poly(NIPA-MAC) particles were added into this medium and mixed immediately. The clotting time was measured by using fibrometer method [25].

Activated Partial Thromboplastin Time (APTT)

Poly(NIPA) and Fe^{3+}-imprinted poly(NIPA-MAC) particles were incubated in 0.1 M phosphate buffer solution (pH: 7.4) for 24 h at room temperature and washed on a glass filter with 0.5 M NaCl solution and distilled water. Fresh frozen pooled human plasma (0.1 mL) was preheated to 37°C for 2 min. The partial thromboplastin (0.3 mL, bioMerieux, Marcy-l'Etoile, France) was also preheated to 37°C for 2 min and was added to preheated human plasma. Then, 10 mg of non-imprinted and imprinted poly(NIPA-MAC) particles were added into this medium. Thirty seconds later, $CaCl_2$ (0.1 mL, 0.025 M) was added, then, the active partial thromboplastin time (APTT) was determined by using the fibrometer method [26].

Prothrombin Time (PT)

In order to determine prothrombin time (PT), one-stage prothrombin method was used [27]. The non-imprinted and the Fe^{3+}-imprinted poly(NIPA-MAC) particles were incubated in 0.1 M phosphate buffer solution (pH: 7.4) for 24 h at room temperature. Fresh frozen pooled human plasma (0.1 M) was preheated to 37°C for 2 min. The thromboplastin (0.2 mL, bioMerieux, Marcy-l'Etoile, France) was also preheated to 37°C for 2 min and was added to preheated human plasma. Then, 10 mg of non-imprinted and imprinted poly(NIPA-MAC) particles were added into this medium. Thirty seconds later, $CaCl_2$ (0.1 mL, 0.025 M) was transferred into the medium. After these operations, the prothrombin time was measured by using fibrometer method [25].

Cell Adhesion Studies

Human blood (heparinized, 500 IU/kg) was contacted with the non-imprinted and the Fe^{3+}-imprinted poly(NIPA-MAC) particles at in-vitro system. It should be noted that prior to the blood contact, polymeric particles were washed with 0.1 M KCl in buffer until no further impurities (monitored by the absorbance at 280 nm) was detected in the washing solution. Polymeric gels were incubated with blood for 1 h. Blood samples were withdrawn at the beginning and at the end of the procedure, and the platelet and leukocyte count of samples were determined by microscopy.

Adsorption-Desorption Studies

Adsorption of Fe^{3+} Ions From Human Plasma

Adsorption of Fe^{3+} ions from thalassemia patient's plasma on the Fe^{3+}-imprinted poly(NIPA-MAC) particles were studied in batch-wise. Fresh human plasma was used in all experiments and obtained from a thalassemic donor. HIV and Hepatitis tests were performed to donor blood samples. Blood samples were centrifuged at 500 g for 30 min at room temperature. Then, thalassemia patient's plasma was incubated with a 100 mg of the Fe^{3+}-imprinted poly(NIPA-MAC) particles at 20°C for 3 h. The concentration of the Fe^{3+} ions in plasma, after the desired treatment periods was measured by using a atomic absorption spectrophotometer (Analyst 800/Perkin Elmer, USA). Deuterium background correction was used and the spectral slit width was 0.5 nm. A hollow cathode Fe^{3+} lamp was used. The instrument response was periodically checked with known Fe^{3+} solution standards. The experiments were performed in replicates of three and the samples were analyzed 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 95% were calculated for each set of samples in order to determine the margin of error.

Selectivity Experiments

In order to show Fe^{3+} (Iron; ionic radius: 64 pm) specificity of the Fe^{3+}-imprinted poly(NIPA-MAC) particles, competitive adsorptions (i.e., Nickel, Ni^{2+}, ionic radius: 69 pm and Cadmium, Cd^{2+}, ionic radius: 97 pm) were also studied. 100 mg/L iron, copper and nickel ions used in aqueous solutions. The Fe^{3+}-imprinted poly(NIPA-MAC) particles were treated with this competitive ions. After adsorption equilibrium, the concentration of Ni^{2+} and Cd^{2+} ions in the remaining solution was measured by AAS.

Distribution and selectivity coefficients of Ni^{2+} and Cd^{2+} with respect to Fe^{3+} were calculated as explained by the following Equation 1:

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

Here, K_d represents the distribution coefficient; C_i and C_f are initial and final concentrations of metal ions, respectively. V is the volume of the solution (mL) and m is the mass of beads used (g).

The selectivity coefficient for the binding of a metal ion in the presence of competitor species (Eq. 2) can be obtained from equilibrium binding data according to (Eq. 3).

$$M_1(\text{solution}) + M_2(\text{sorbent}) <=> M_2(\text{solution}) + M_1(\text{sorbent}) \quad (2)$$

$$k = ([M_2]_{solution} \times [M_2]_{sorbent}) / ([M_1]_{solution} \times [M_2]_{sorbent}) = K_d\,(Cd^{2+}) / (K_d(X^{2+}) \quad (3)$$

where k is the selectivity coefficient and X^{2+} represents Ni^{2+} and Cd^{2+} ions. A comparison of the k values of the imprinted beads with those metal ions allows an estimation of the effect of imprinting on selectivity.

A relative selectivity coefficient k' (Eq. 4) can be defined as

$$k' = k_{imprinted}/k_{control} \quad (4)$$

Desorption and Repeated Use

Desorption of Fe^{3+} ions were studied 0.1 M nitric acid (HNO_3) solution. The Fe^{3+}-imprinted poly(NIPA-MAC) particles were placed in this desorption medium and stirred continuously (at a stirring rate of 400 rpm) for 1 h at room temperature. The final Fe^{3+} ions concentration in the desorption medium was measured by atomic adsorption spectrometer.

In order to test the reusability of the non imprinted and the Fe^{3+} imprinted poly(NIPA-MAC) particles, Fe^{3+} ions adsorption-desorption procedure was repeated five times by using the same polymeric sorbent. In order to regenerate and sterilize, after desorption; the gels were washed with 50 mM NaOH solution.

RESULTS AND DISCUSSIONS

Characterization of Poly(NIPA-MAC) Gels

Poly(NIPA-MAC) gels have hydrophilic character, so they would swell in aqueous media. The swelling rate of poly(NIPA-MAC) has decreased with introducing MAC monomer into the poly(NIPA) structure. The poly(NIPA-MAC) has about 11.81% swelling ratio within 240 min, whereas the poly(NIPA) has about 12.56% within the same time frames. Template removed Fe^{3+}-imprinted poly-(NIPA-MAC) has about 10.87% swelling ratio within 240 min, whereas non-imprinted poly(NIPA-MAC) has about 11.81% within the same interval.

Usually, the volume phase transition temperature or LCST of these hydrogel is defined as the temperature at which the swelling ratio has decreased to a half of its value at the initial temperature or room temperature [28]. The hydrogel`s LCST is also regarded as the temperature at which phase-separation degree (changes of the swelling ratio vs. temperature changes around the transition temperature, $\Delta SR/\Delta T$) is greatest or the temperature at which the swelling ratio of hydrogel decreased most dramatically [29,30]. The poly(NIPA) gels showed a LCST temperature about 32°C which was matching with the literature [31]. Introducing MAC residue into the polymer structure, the LCST temperature increased from 32°C to 34°C and 36°C, at non-imprinted poly(NIPA-MAC) and template removed Fe^{3+}-imprinted poly(NIPA-MAC) gels, respectively. The highest LCST temperature (36°C) was observed at template removed Fe^{3+}-imprinted poly(NIPA-MAC) gels, which may be concluded as effect of remaining Fe^{3+} ions after removal of template on the inhibition of shrinking of the gels is higher than the non-imprinted particles by interaction of the Fe^{3+} ions with water molecules. Poly(NIPA), non-imprinted poly(NIPA-MAC) and template removed Fe^{3+}-imprinted poly(NIPA-MAC) particles exhibit a negative temperature sensitive, which is swelling at lower temperature and shrinking at higher temperature. Under equilibrium swelling conditions, all gels showed increasing swelling at lower temperatures, but they deswelled at high temperatures because of the aggregation of the network chains. When the external temperature was increased from 5 to 60°C, the volume or water content inside gels decreased slowly during the shrinkage, and the water release rate is controlled mainly by collective diffusion of the hydrogel [32].

The molecular formula of synthesized MAC co-monomer and MAC-Fe^{3+} complex is shown in Figure 1. FTIR spectrum of MAC has the characteristic stretching vibration carboxyl–carbonyl, amide I and amide II absorption bands at 1607 cm^{-1}, 1533 cm^{-1} and 1453 cm^{-1}, respectively (Figure 2A). The S–H bending peak appears at 2625 cm^{-1} of MAC. For the characteristic determination of complex, due to linear coordinate covalent complex formation, the S-

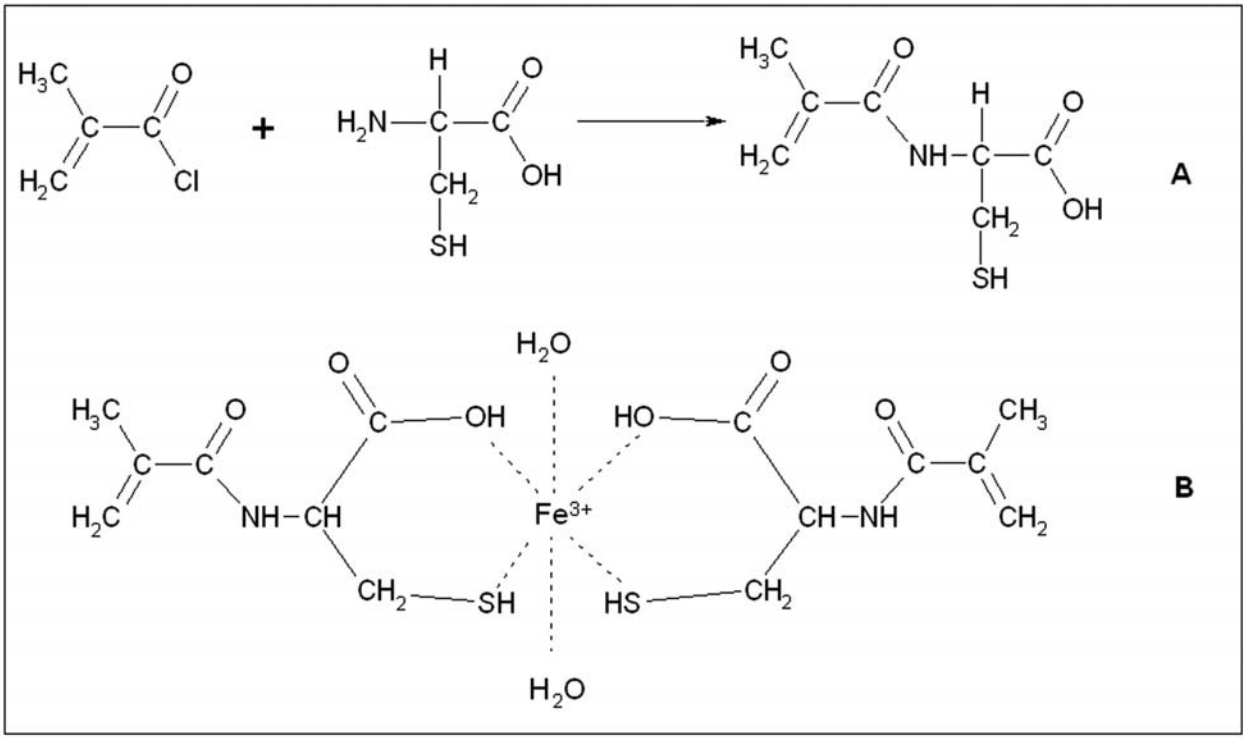

Figure 1. The molecular formula of (A) MAC monomer; (B) MAC Fe^{3+}complex.

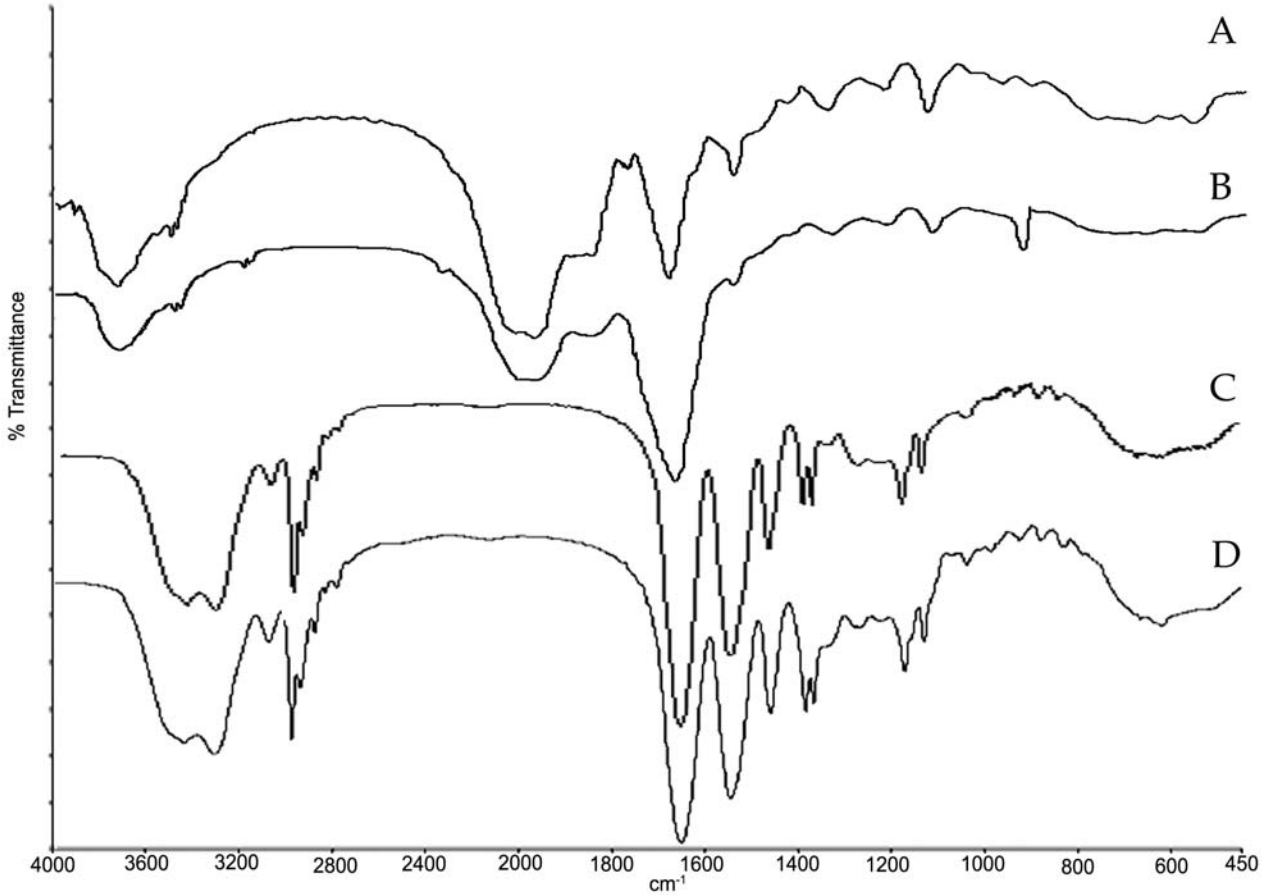

Figure 2. FTIR spectra of MAC monomer (A), MAC- Fe^{3+} complex (B), non-imprinted poly(NIPA-MAC) (C) and poly(NIPA-MAC)-Fe^{3+} complex (D).

H bending peak at 2625 cm^{-1} slips to the down field at 2514 cm^{-1}, as a result of decreasing the electron density of sulphydryl of MAC monomer (2B).

Then, MAC–Fe^{3+} complex were polymerized with NIPA monomer by free radical polymerization. The FTIR spectrum of non-imprinted poly(NIPA-MAC) 2C) and Fe^{3+}-imprinted poly(NIPA-MAC) (Figure 2D) particles showed a broad band in the range of 3600–3200 cm^{-1}, which belongs to N–H stretching vibration of the poly(NIPA). The typical amide I band (1648 cm^{-1}), consisting of C=O stretch of poly(NIPA) and amide II band (1541 cm^{-1}), including N–H vibration were evident in both spectrum. The C-S peak of MAC monomer in the structure of poly(NIPA-MAC) around 620 cm^{-1} was disappeared in the case where NIPA monomer was polymerized with MAC-Fe^{3+} complex because of decreasing the electron density in C-S interaction [33].

^{1}H-NMR was used to determine the synthesis of MAC structure. ^{1}H-NMR spectrum is shown to indicate the characteristic peaks from the groups in MAC monomer. These characteristic peaks are as follows: ^{1}H-NMR (DMSO): 7.67-7.36 ppm belongs to SH and –NH protons. They could not be observed in H_2O because of being mobile protons. Peaks at 5.67 and 5.31 ppm indicate ethylene, 4.16 ppm –CH and 1.88 ppm -CH_3.

Blood Compatibility Studies

A biomaterial is a substance that is used in medical devices or in prostheses designed for contact with the living body for an intended method of application and for an intended period. Synthetic polymers are the most diverse class of biomaterials. Polymeric biomaterials are widely used in both medical and pharmaceutical applications [34]. These applications include a variety of implants or other supporting materials (e.g. vascular grafts, artificial hearts,

intraocular lenses, joints, mammary prostheses and sutures), extracorporeal therapeutic and other supporting devices (e.g. hemodialysis, hemo-perfusion, blood oxygenation and bags), controlled release systems and clinical diagnostic assays (mainly as carriers) [35]. All biomaterials must meet certain criteria and regulatory requirements before they can be qualified for use in medical applications. Depending on the intended end-use, a biomaterial may be subjected to a set of tests, such as blood-compatibility, tissue-compatibility, carcinogenicity, mutagenicity, biodegradation and mechanical stability [27].

When biomaterials in use come into contact with blood, first small molecules (e.g. water and ions) reach to the surface which may or may not be adsorbed. This is followed by plasma protein adsorption. The first protein layer adsorbed on the biomaterial surface determines the subsequent events of the coagulation cascade (via the intrinsic pathway), and the complement activation (via the intrinsic-extrinsic pathways) [36].

Coagulation Times

In order to estimate the blood-compatibility of the non-imprinted poly(NIPA-MAC) and the Fe^{3+}-imprinted poly(NIPA-MAC) particles, in-vitro coagulation times (CT), activated partial thromboplastin time (APTT) and prothrombin time (PT) tests were carried out. It should be mentioned that APTT tests exhibit the bioactivity of intrinsic blood coagulation factors and PT test relates to extrinsic blood coagulation factors on biomaterial surface. CT test shows in-vitro coagulation time.

Table 2 summarizes the coagulation data obtained in these tests. As can be seen from Table 2, all the clotting times for the non-imprinted poly(NIPA-MAC) and the Fe^{3+}-imprinted poly(NIPA-MAC) particles were lower than control. But these decreases are tolerable by the body. Therefore, we concluded that the blood-compatibility of newly synthesized non-imprinted poly(NIPA-MAC) and Fe^{3+}-imprinted poly(NIPA-MAC) particles were rather good, and all the clotting times were quite reproducible comparing with the values reported in the related literature. Consequently, in this article, the incorporation of MAC as a co-monomer may exert beneficial effects in two ways. First, improved blood-compatibility of the particles will reduce adverse body reactions to possible biomedical treatment applications (i.e., extracorporeal therapy), and second, reductions in non-specific adsorption will reduce undesirable losses of beneficial proteins from treated blood [37].

Table 2. Coagulation times of human plasma (reported in sec).

Experiments	(APTT)	(PT)	(CT)
Control Plasma	82.4	38.5	289
Non-imprinted poly(NIPA-MAC)	78.9	36.3	276
Imprinted poly(NIPA-MAC)	78.5	36.0	278

Cell Adhesion Studies

Table 3 summarizes hematological data obtained from in-vitro blood assay. Loss of platelet with the non-imprinted poly(NIPA-MAC) and the imprinted poly(NIPA-MAC) particles were 1.1% and 1.6%, respectively. Lost of leukocyte with the non-imprinted poly(NIPA-MAC) and the imprinted poly(NIPA-MAC) particles were 3.8% and 5.7%, respectively. As seen here, there is no significant cell adhesion on the particles. These observations

Table 3. Platelet and leukocyte adhesion with non-imprinted poly(NIPA-MAC) and the imprinted poly(NIPA-MAC) particles .

Substance	Platelet (x 10^{-3}/mm^3)		Leukocyte (x 10^{-3}/mm^3)	
	Initial/ Final	Loss (%)	Initial/ Final	Loss (%)
Non-imprinted poly(NIPA-MAC)	430/420	1.1	5.2/5.0	3.8
Imprinted poly(NIPA-MAC)	430/418	1.6	5.2/4.9	5.7

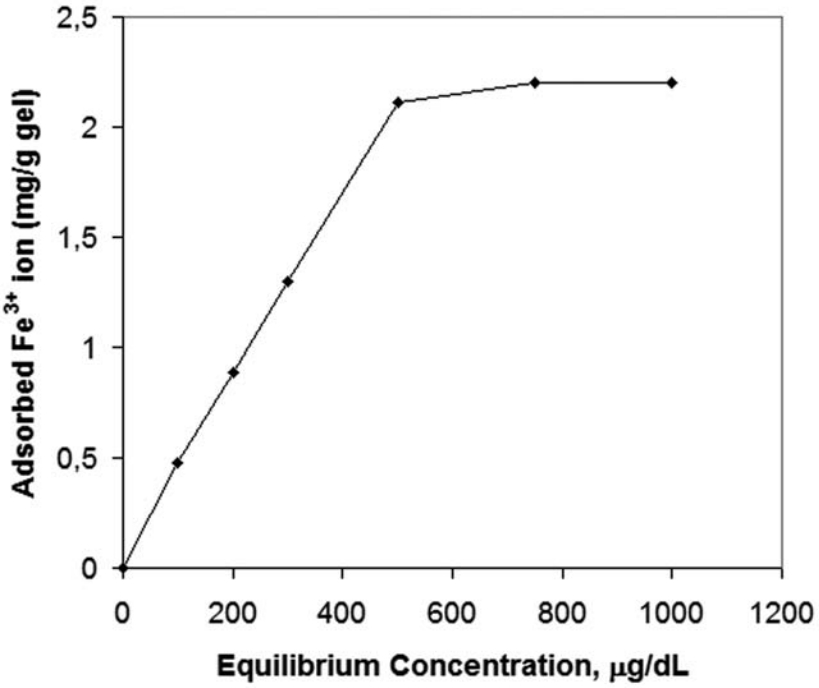

Figure 3. Fe^{3+} adsorption from thalassemia patient's plasma by using Fe^{3+}-imprinted poly(NIPA-MAC) gels. The effect of ion concentration.

showed that surfaces of the particles are resistant to adhesion of platelets and leukocytes. In conclusion, because of the good non-thrombogenic properties, these gels seem to be very promising affinity adsorbents for biomedical applications such as an extracorporeal adsorption therapy.

Adsorption-Desorption Studies

Adsorption of Fe^{3+} ions from thalassemia patient's plasma

Effect of Fe^{3+} ions concentration

The results obtained from the adsorption experiments of Fe^{3+} ions at different equilibrium concentrations are summarized in Figure 3. The adsorption values increased with increasing Fe^{3+} ions, and a saturation value is achieved at Fe^{3+} ion concentration of 1000 µg/dL, which represents saturation of active binding cavities on the Fe^{3+}-imprinted poly(NIPA-MAC) particles. The increase in the adsorption amount of Fe^{3+}-imprinted poly(NIPA-MAC) particles may be indicative of the formation of new binging sites that are not present in the poly(NIPA-MAC) particles. The maximum adsorbed amount of Fe^{3+} ions on the Fe^{3+}-imprinted poly(NIPA-MAC) particles was found to be 2.2 mg/g.

Selectivity Experiments

Competitive and selectivity adsorption studies performed in two ways. First, adsorption studies were done by using 100 mg/L of Fe^{3+}, Ni^{2+} and Cd^{2+} solutions separately. Second, adsorption studies were done in the mixture of these metals at 100 mg/L. Cd^{2+} and Ni^{2+} were chosen as competitive

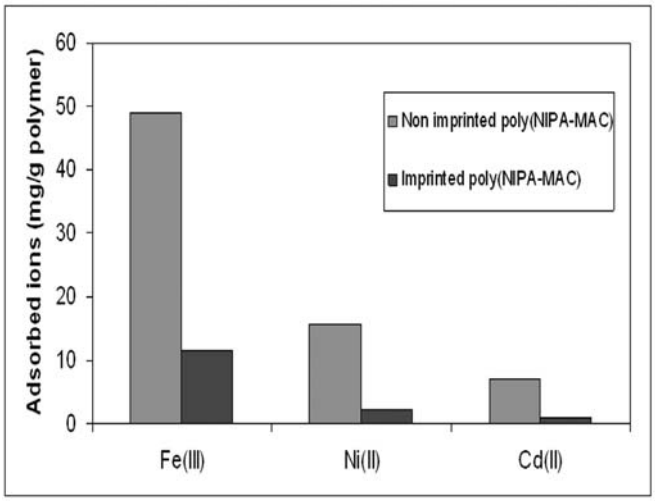

Figure 4. Adsorbed Fe^{3+}, Cd^{2+} and Ni^{2+} ions both non-imprinted and Fe^{3+}-imprinted poly(NIPA-MAC) particles in separate solutions. 10 mL, 100 mg/L solution, pH: 5.0, 0.01 g polymer, T: 20°C.

metal ions because of their similar ionic radius. Figure 4 and 5 show adsorbed template, Cd^{2+} and Ni^{2+} ions both in imprinted and non-imprinted particles in separate solutions and mixture, respectively.

The Fe^{3+} adsorption capacity of the non-imprinted and the imprinted poly(NIPA-MAC) particles was much more higher than Ni^{2+} and Cd^{2+} ions when adsorption studies were performed in each ion solution. This can be concluded that, of non-imprinted and imprinted poly(NIPA-MAC) particles show the following metal ion affinity in the order of $Fe^{3+} > Ni^{2+} > Cd^{2+}$. In order to show the competitive adsorption, a mixture of Fe^{3+}, Ni^{2+} , Cd^{2+} ions at 100 mg/L concentration was used. Under competitive conditions, less amount of Fe^{3+}, Ni^{2+}, Cd^{2+} ions adsorbed to the non-imprinted and the imprinted poly(NIPA-MAC) particles because of competitions of these ions. However, less amount of Ni^{2+} and Cd^{2+} ions adsorbed to the particles and selectivity increases.

Figure 5 shows adsorbed template and Cd^{2+} and Ni^{2+} ions both in imprinted and non-imprinted particles in mixture. Table 4 summarizes K_d, and k, values of Cd^{2+} and Ni^{2+} with respect to Fe^{3+}.

A comparison of the Kd values for the Fe^{3+} the imprinted poly(NIPA-MAC) samples with the control samples show an increase Kd for Fe^{3+} while Kd decrease for Ni^{2+} and Cd^{2+}. The relative selectivity coefficient is an indicator to express metal adsorption affinity of recognition sites to the imprinted Fe^{3+} ions. These results show that relative

Table 4. K_d, k, and *k'* values of the non-imprinted and imprinted particles.

Metal ion	Non-imprinted		Imprinted		
	K_d	k	K_d	k	k'
Fe^{3+}	0.100		0.180	-	-
Ni^{2+}	0.02	1.05	0.138	4.75	4.52
Cd^{2+}	0.01	1.96	0.071	9.43	4.81

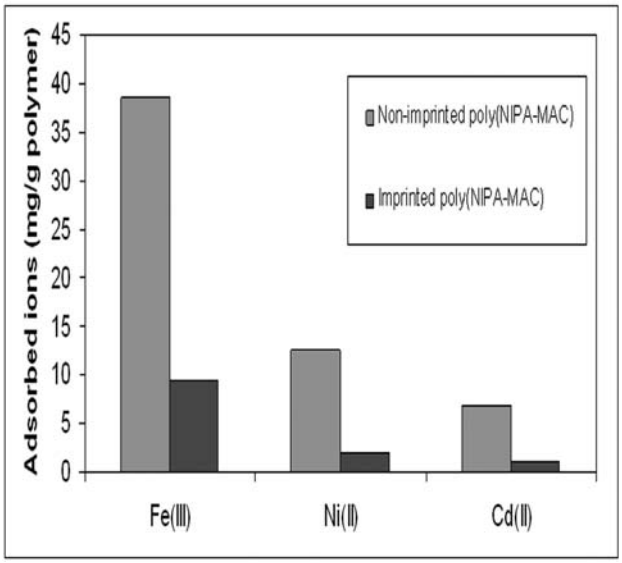

Figure 5. Adsorbed Fe^{3+}, Cd^{2+} and Ni^{2+} ions both non-imprinted and Fe^{3+}-imprinted poly(NIPA-MAC) particles in competitive solutions. 10 mL, 100 mg/L solution, pH: 5.0, 0.01 g polymer, T: 20°C.

selectivity coefficients of imprinted beads for $Fe^{3+}/Ni^{2+}/Cd^{2+}$ 4.52 and 4.81 times greater than non-imprinted particles, respectively (Table 4). From these results, it can be said that the particles imprinted with Fe^{3+} ions indicates the selectivity for the Fe^{3+} ion as expected.

Desorption and Repeated Use

The regeneration of the adsorbent is likely to be a key factor in improving process economics. Desorption of the Fe^{3+} ions from the non-imprinted and Fe^{3+}-imprinted poly(NIPA-MAC) particles was performed in a batch experimental set-up. Various factors are probably involved in determining rates of Fe^{3+} desorption, such as the extent of hydration of the metal ions and polymer microstructure. However, an important factor appears to be binding strength. In this study, the desorption time was found to be 60 min. Desorption ratios are high (up to 73% and 69% for the non-imprinted and the Fe^{3+}-imprinted poly(NIPA-MAC) particles respectively). In order to obtain the reusability of the non-imprinted and the Fe^{3+}-imprinted poly(NIPA-MAC) particles, adsorption-desorption cycles were repeated 5 times by using the same imprinted beads. The adsorption capacity of the recycled non-imprinted and Fe^{3+}-

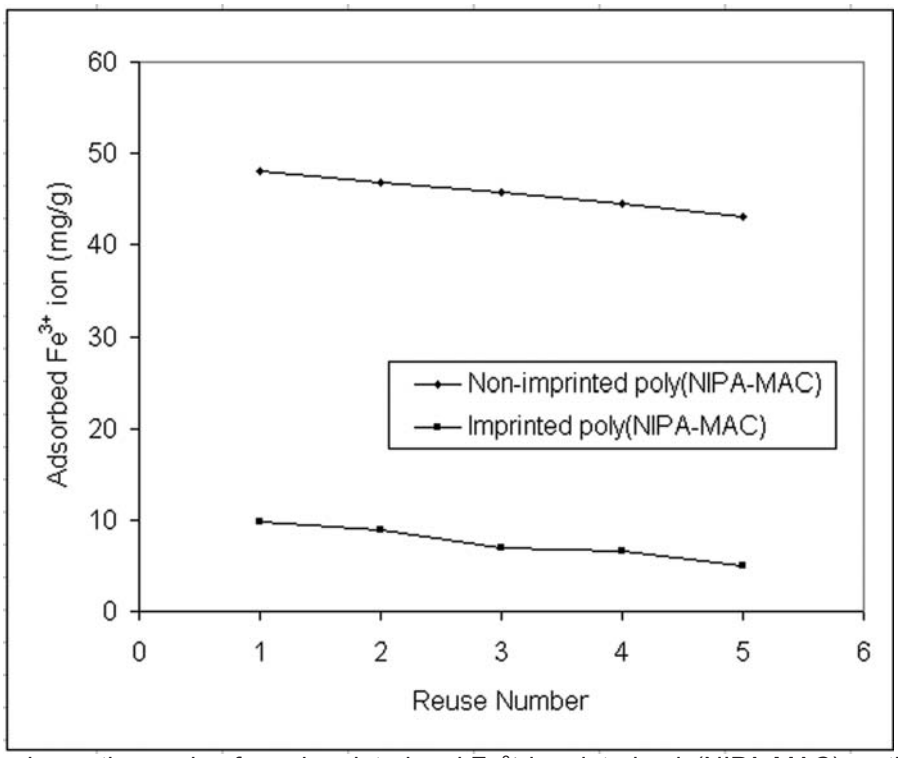

Figure 6. Adsorption-desorption cycle of non-imprinted and Fe^{3+}-imprinted poly(NIPA-MAC) particles.

imprinted poly(NIPA-MAC) particles can still be maintained at level 73% and 69% at the 5th cycle (Figure 6). It can be seen concluded that the non-imprinted and Fe^{3+}-imprinted poly(NIPA-MAC) particles can be used many times without decreasing their adsorption capacities significantly.

CONCLUSION

Considerable researches have been focused on polymeric materials those change their structure and functions responding to external physical, chemical, and electrical stimuli (light, temperature, pH, substance concentration, solvent composition, and electric fields, etc.). Intelligent materials embodying these concepts might contribute to establish fundamental principles for fabrication of novel systems. Thermosensitive gels have attracted a great deal of attention for the applications to drug delivery systems. Novel adsorbents using thermosensitive gels for trapping heavy metals were reported several papers [8-11], where poly(NIPA) was used as a thermosensitive backbone polymer. A chelating group, which interacts with heavy metals, was introduced into poly(NIPA) with a molecular imprinting technique [12] using a specific metal as the template. The molecular imprinted adsorbents reconstruct multi-point adsorption sites at a specific temperature and disrupt them through swelling deformation at a lower temperature. The adsorbents, therefore, are suitable for the temperature swing adsorption (TSA), i.e. the control of adsorption and desorption of a specific heavy metal with the change in temperature. Thalassemia is the world's most common hereditary disease, and is a paradigm of monogenic genetic diseases [22]. Because of increased population mobility, the disease is found today throughout the world, even in places far from the tropical areas in which it arose. Therapy of thalassemia has in the past been confined to transfusion and chelation [23]. Recently, novel modes of therapy have been developed for thalassemia, based on the pathophysiology and molecular pathology of the disease, both of which have been extensively studied. The therapeutic modalities currently in use for the supportive treatment of thalassemia, both those that are standard therapy and those that are in clinical trials are

such as; transfusion, chelation (intravenous and oral), antioxidants and various inducers of fetal hemoglobin (hydroxyurea, erythropoietin, butyrates, hemin) [24]. Most of the newer therapies are suitable primarily for thalassemia intermedia patients. In present study, we prepared ion-imprinted polymeric gels, which were used for the selective removal of Fe^{3+} ions from thalassemia patient`s plasma. Adsorption of $Fe^{3+}$ ions from thalassemia patient's plasma were studied in batchwise. The results show that $Fe^{3+}$ imprinted gels can be used for selective adsorption of the ion from thalassemia patient`s plasma.

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