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Research Article

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Designing of Alternative Polymeric Nano-Chelator for Treatment in Acute Iron Poisoning by Molecular İmprinting Approach

Akut Demir Zehirlenme Tedavileri için Moleküler Baskılama Yaklaşımı ile Alternatif Polimerik Nano Şelatörlerin Tasarlanması

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ABSTRACT

The aim of this study is to develop an alternative polymeric chelating agent for rapid and selectively removal with high capacity of iron $(Fe^{3})^{+}$ ions from the gastrointestinal (GI) tract for the oral treatment of acute iron poisoning. For this purpose, Fe^{3+} imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-(l)- glutamic acid) (HEMA-MAGA) nanoparticles were synthesized by surfactant free emülsiyon polymerization. Molecular imprinting (MIP) technique was used to enhance the selectivity of nanoparticles. Due to being carboxyl and amide groups on the MAGA monomer, it was chosen as a chelating agent for Fe^{3+} ions. Before the synthesizing of Fe^{3+} imprinted polymer, Fe^{3+} ions were complexed with (N-methacryloyl-(l)-glutamic acid) MAGA and then Fe^{3+} imprinted nanoparticles were synthesized in the presence of this Fe^{3+}-MAGA complexes. Poly(HEMA-MAGA) nanoparticles were characterized by infrared spectroscopy (FTIR), atomic force microscopy (AFM). Average particle size and size distribution also determined by zeta sizer. The specific surface area and mead diameter of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was 895 $m^2.g^{-1}$ and 95.3 nm, respectively. The maximum Fe^{3+} ions binding capacity of the poly(HEMA-MAGA) nanoparticles at pH:4.0 were 206.4 $mg.g^{-1}$ nanoparticles in intestinal mimicking solution(IMS). Fe^{3+} removal performance of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles with presence of other ions, optimum medium pH, temperature and equilibrium binding time were also investigated. Fe^{3+} removal studies were performed in both aqueous solution and intestinal mimicking solution. The results indicate that Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles is an alternative chelating agent for the selective Fe^{3+} ions removal in a short time and with very high capacity.

Key Words

Acute iron poisoning, iron removal, molecularly imprinted polymers (MIP), iron imprinting, glutamic acid.

ÖZ

Bu çalışmanın amacı akut demir zehirlenmelerinin tedavisi için, sindirim sisteminden demir iyonlarının yüksek kapasitede ve hızlı bir şekilde uzaklaştırılmasına yönelik alternatif polimerik şelatlayıcı geliştirilmesidir. Bu amaçla surfaktansız emülsiyon polimerizasyon yöntemi ile Fe^{3+} iyonları baskılanmış poli(hydroxyethyl methacrylate-N-methacryloyl-(l)- glutamic acid) (HEMA-MAGA) nanopartikülleri sentezlendi. Moleküler baskılama yöntemi nanopartiküllerin seçiciliğini artırmak için kullanıldı. Karboksil ve amid grupları olması nedeniyle MAGA monomeri Fe^{3+} iyonlarını şelatlayıcı ajan olarak seçildi. Fe^{3+} iyonları baskılanmış polimer sentezlenmeden önce Fe^{3+} iyonları MAGA monomeri ile kompleksleştirildi ve sonra Fe^{3+}-MAGA kompleksinin varlığında Fe^{3+} baskılanmış nanopartiküller sentezlendi. Poli(HEMA-MAGA) nanopartiküller infrared spektroskoisi (FTIR), atomik kuvvet mikroskopisi (AFM) ve partikül boyut ve boyut dağılımıda zeta sizerla karakterize edildi. Fe^{3+} baskılanmış poli(HEMA-MAGA) nanopartiküllerin yüzey alanı ve ortalama çapı sırasıyla 895 $m^2.g^{-1}$ ve 95.3 nm dir. Poli(HEMA-MAGA) nanopartiküllerin maksimum Fe^{3+} iyonları bağlama kapasiteleri yapay mide suyunda pH: 4.0 te 206.4 $mg.g^{-1}$ nanopartiküldür. Fe^{3+} baskılanmış poli(HEMA-MAGA) nanopartiküllerin Fe^{3+} iyonları uzaklaştırma performansları başka iyonlar varlığında, optimum ortam pH'ı, sıcaklık ve denge bağlanma zamanı incelendi. Fe^{3+} iyonları uzaklaştırma çalışmaları hem sulu çözeltide hemde yapay mide suyu çözeltisinde yapıldı. Yapılan tüm bu deneysel çalışmaların sonuçları Fe^{3+} baskılanmış poli(HEMA-MAGA) nanopartiküllerin Fe^{3+} iyonlarının çok kısa zaman içerisinde ve çok yüksek kapasitede uzaklaştırmada alternatif şelatlayıcı ajan olabileceğini göstermektedir.

Anahtar Kelimeler

Akut demir zehirlenmesi, demir uzaklaştırılması, moleküler baskılanmış polimerler, demir baskılama, glutamik asit.

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INTRODUCTION

Iron is a trace element, and plays several important roles in human body. Iron is the component of metaloprotein and cofactor of some enzymes as well as it participates essential biochemical activities such as oxygen transport, electron transfer, catalysis and energy metabolism. Iron also participates in many biochemical, metabolic and biological process. Human body contains 5 gr iron and 10 mg iron is absorbed in duodenum and upper jejunum by diets, daily [1,2].

Although iron has very important function in body, all over the world many people suffer from iron deficiency, especially women lost iron during menstrual cycles. Consumption of inadequte iron containing diet cause to iron deficiency or anemia. In order to prevent or treatment of iron deficiency or anemia, iron containing supplements are used. Generally many supplements are taken orally as a tablets, which are mostly contain iron (II) sulfate. Due to the common usefulnes, this iron tablets are available without prescription and kept at many home. Produced iron supplement for compensation of iron deficiency can looks like candy because they brightly colored and sugar coated. So they are attract children attention. Many children reach easly this supplement or iron containing vitamin and syrup. Mostly, a vitamin or mineral suplement tablet contains 60 mg of elemental iron [3].

Like inadequate uptake, excess intake of iron is also problem for human. Because there is no regulation mechanism for excess iron excretion from the body. Ingestion of 60 $mg.kg^{-1}$ body weight of iron or serum iron levels more than 500 $\mu g.dL^{-1}$ can cause toxicity, and may be lethal for children. It can cause serious risk of acute liver failure and eventually death. Iron is corrosive to the gastrointestinal mucosa and high blood level of free iron ions can react with peroxides to produce free radicals, which are highly reactive and can damage to DNA, proteins, lipids other cellular components and than promote cell death and tissue injury, finally organ failure especially liver. Non bounded iron ions on the transferin accumulate primarily in the liver, spleen, heart, kidneys and lungs [4].

Iron poisoning can be acute or chronic. Chronic iron poisoning is caused by result of accumulation of iron in body because of frequently blood transfusions of in B thalassemia, hereditary hemochromatosis and sickle cell anemia patients [5].

Since humans do not have an iron excretion pathway, excess iron is eliminated from the body with chelating agents. For the chronic iron poisoning treatment, desferrioxamine (DFO), deferiprone (L1) and desferasirox (ICL-670), which are clinically approved iron chelators, are used [6].

A desferrioxamine as the most common drug, is an iron chelating agent, and used since 1960. However, DFO with the longest clinical experience in treating iron poisoning, has some drawback such as its high cost, lack of oral efficiency and major side-effects in the long term neurotoxicity, hypotension, short plasma half life and must be administered by long and frequent subcutaneous infusions (12-24 h/5-6 days/week). There is no orally effective alternative to deferoxamine in the treatment of iron poisoning [7,8].

Acute iron poisoning may be caused by suicidal or accidental ingestion of large amount of iron, either as tablets of iron salts or as constituents of vitamin and mineral supplements or syrups.

In recent years, iron poisoning increased because of wide spread use of iron containing supplements. According to the 2015 Annual Report of the American Association of Poison Control Centers' (AAPCC) National Poison Data System; 4072 single exposures to iron or iron salts, with one major outcome and one death. And, the reports show 2036 case occured in children younger than 5 years, and 3211 were unintentional ingestion [9]. Acute iron poisoning may be cause hemorrhagic nausea vomiting, diarrhea, abdominal pain, hypovolemia or shock acidosis, failure of organs such as liver, heart and kidneys, coma, cardiovascular collapse, and eventually death [10].

The treatment of acute iron poisoning is initiated with gastric lavage and whole bowel irrigation than chelation therapy. Gastric lavage and bowel irrigation should be used as soon as possible to reduce of intestinal absorption of toxic iron ions in 0-6 hours [11,12].

In treatment of acute iron poisoning, early recognition is very important to reduce the effects of poisoning and to prevent morbidity and mortality. Therefore, designing an orally active chelating agent for preparing of lavage solutions are gaining importance. Because the intestinal absorbtion of swallowed iron can be blocked with using oral active chelating agent than remove ex-

cess iron ions through the faecal excretion routes. Altough activated carbon is also commonly used as the adsorbent in poisoning treatment, but it does not adsorb iron ions [13,14].

Many researcher have been studied to develop alternative chelators such as covalently attached DFO to dextran and hydroxyethyl starch (HES), degradable PEG based copolymers, hydroxamic acid group containing hydrogels, dendrimeric iron chelators and polymer based chelators for treatment acute iron poisoning. Therefore, several techniques and chelation therapies have been investigated [15-20]. Among them, polymeric chelating agents with their superior feature because of non-toxicity, high affinity and specifty to iron ions, biocompatibility, forming fast and insoluble complex, preventing the absorption of Fe^{3+} ion in GI tract, promoting the removal of iron and orally active is the most promising adsorbent [21-29].

Molecular imprinting is a method that used in the synthesis of polymeric structures with the high selectivity and specifity towards the target molecules or ions [30]. Polymerization is performed in the presence of the target and functional monomer. After removal of the target molecule from the polymeric matrix, three dimensional (3D) chemically selective binding sites also stated synthetic receptors are obtained. In molecular imprinting method, secondary interactions such as hydrogen bonding, electrostatic or hydrophobic interactions play an important role [30]. Molecular imprinted polymers because of their unique feutures like being easy to prepare, stable, cost effectiveness and capable of molecular recognition have recieved much attention in various field such as biotechnology, environmental, sensors, medicine, etc. [31-35]. Synthetic receptors obtained via MIP technology are not affected by harsh condition such as high temperature, pH and organic solvent when comparating biological receptors. Nanoparticle has been receiving attention as an adsorbent due to its large surface-to-volume ratios and unique chemical and physical properties [36]. The large surface area of the nanoparticles can increase their adsorption capacities and reduce adsorption time, which are very important advantages because of reaching to high adsorption capacity in a short time. Therefore, it is useful for the removal of metal ions from varios medium in comparation with conventional adsorbents. Polymeric nanoparticles is the most promising alternative of the conventional materials, which are used in separation and purification technology as well as medical imaging, drug delivery, etc. [37,38].

In this study, HEMA based polymeric nanoparticles were synthesized for the treatment of acute iron poisoning. Backbone of poly(HEMA-MAGA) nanoparticles carring glutamic acid increase biocompatiblity of polymer. MAGA was selected as Fe^{3+} complexing monomer. Because primary amine groups and pendant carboxy groups of MAGA form complexes with Fe^{3+} ions. Strong complex formation occurs between carboxylic groups and iron ions. By using mip technique, selectivity of polymeric chelating agent to Fe^{3+} ions was enhanced. Due to the their nano size, prepared nanoparticles have high adsorption capacity and reaches maximum adsorption capacity in a very short time. Our strategy is to remove Fe^{3+} ions before absorption of intestinal system with using chelating agent via orally treatment. After adsorption of the Fe^{3+} ions onto the nanoparticles from the gastrointestinal tract system, removal of excess iron will come true through the faecal excretion routes.

EXPERIMENTAL

Chemicals

L-Glutamic acid hydrochloride and methacryloyl chloride, potassium persulfate (St Louis, USA) were purchased from Sigma (St. Louis, MO USA). Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) were obtained from Fluka A.G. (Buchs, 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). Stock solution of 1000 $mg.L^{-1}$ Fe^{3+} was prepared by dissolving iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) (Merck, Darmstadt, Germany). Standard iron solutions were prepared daily by dilution of the stock solutions. Barnstead (Dubuque, IA, USA) ROpure LP reverse osmosis unit of deionized water was used in the all experiments.

Preparation of Fe^{3+}-MAGA complex and Fe^{3+}imprinted nanoparticles

Firstly, MAGA monomer was synthesized, then MAGA-Fe^{3+} complexes was formed, and finally Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were prepared. The surfactant free emulsion polymerization method was used for the preparation of Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles. A preparation procedure is described below. The synthesis of MAGA monomer was

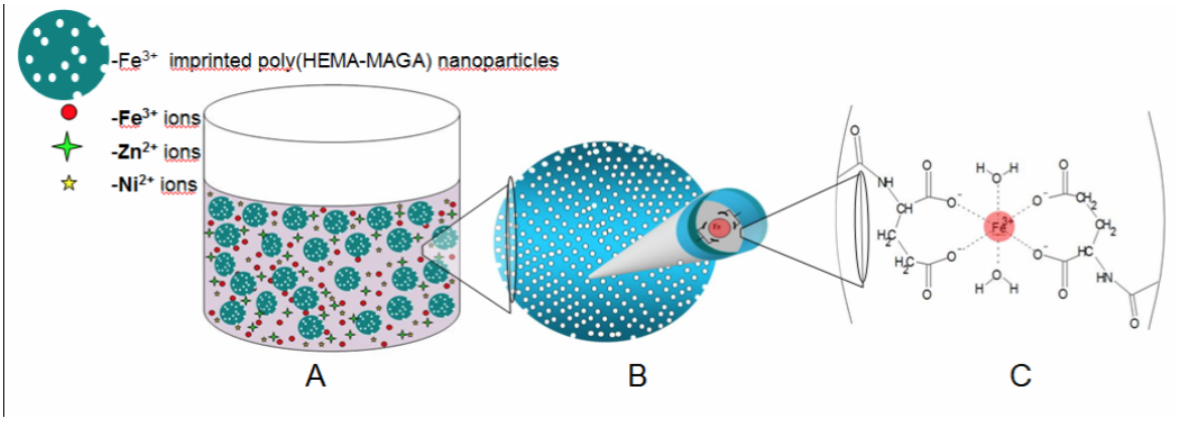

Figure 1. Schematic presentation of experiments. A) Fe^{3+} binding medium of nanoparticles, which contain competitive ions Zn^{2+} and Ni^{2+} B) A single Fe^{3+} imprinted nanoparticles with tailored cavities for Fe^{3+} ions C) Complex formation of carboxylic groups and Fe^{3+} ion.

described as the procedure reported by elsewhere [39].The MAGA-Fe^{3+} complex monomer, was prepared by addition of solid N-methacryloyl-L glutamic acid (MAGA) (0.430 g, 2.0 mmol) into 15 mL solution of ethanol- water mixture (50/50, v/v) in a vessel, followed by dissolution of iron nitrate ($Fe(NO_3)_3 \cdot 9H_2O$) (0.404 g,1.0 mmol) at room temperature by constant stirring (250 rpm) for 3 h. Then, the formed monomer-metal complex (MAGA -Fe^{3+}) was filtered, washed with 96% ethanol, and dried in a vacum oven at 40°C for 24h. After formation of MAGA-Fe^{3+} complexes, Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were synthesized.

Preparation procedure for polymeric Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles is given below. Briefly, the stabilizer, poly(vinyl alcohol) (PVAL, 0.5 g), was dissolved in 50 ml deionized water for the preparation of the dispersion phase. Organic phase including HEMA/EGDMA/Fe-MAGA complex mixture 0.6 ml/0.2 ml/300 mg respectively stirred about 30 min at room temperature then, transferred into the dispersion medium, which was placed in a sealed pyrex polymerization reactor (volume: 250 mL), in a thermostatic water bath. KPS (0.5 $mg.ml^{-1}$) used as an initiator, was added polymerization solution and mixed in an ultrasonic bath for 30 min. Polymerization was carried out in a shaking bath with constant temperature at 70°C, for 6 h. Non-imprinted poly(HEMA-MAGA) nanoparticles were prepared in the same way, without addition of MAGA-Fe^{3+} complexes. After polymerization, Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were separated from the polymerization medium by the precipating with the a centrifuge (Zentrifugen, Universal 32 R, Germany) of 25000 g for 30 min. Then, it was resuspended in ethanol and water solutions, and washed with ethanol and water several times.

After cleaning procedure, the template Fe^{3+} ions were removed from the polymeric nanoparticles by using 0.1 M EDTA solution**.** The MIP nanoparticles were added into 0.1 M EDTA solution for 24 h at room temperature and with 200 rpm apparatus. This procedure was repeated several times until the template molecule (i.e. Fe^{3+} ions) could not be detected in the cleaning solutions with inductively coupled plasma-optical emission spectrometer (ICP-OES). Finally, the template free nanoparticles were suspended in deionized water. By using Freeze dryer (lyophilizator), and Fe^{3+} imprinted nanoparticles get obtained as dry powders, and kept in refrigerator.

Characterization of nanoparticles

The average size and size distribution of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were determined by Nano Zetasizer Nano ZS (NanoS, Malvern Inst. London, UK). Poly(HEMA-MAGA) nanoparticles were dispersed in 3ml deionized water and placed onto the sample holder. The light scattering was performed at an incidence angle of 90° and 25°.

The shape and size of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were analyzed by atomic force microscopy (AFM) (Nanomagnetics Instruments, Oxford, UK) in tapping mode. Samples were scanned with 2 $\mu m.s^{-1}$ scanning rate at 256×256 pixels resolution. Scanning was performed at a scan rate of 1.001 Hz and scan size of 5000 µm. The tip loading force was minimized to avoid structural changes of the sample

FTIR spectra of the samples were obtained using a FTIR spectrophotometer (Thermo Fisher Scientific, Nicolet iS10, Waltham, MA, USA). The dry nanoparticles (about

5 mg) were mixed with KBr (0.1 g, IR Grade, Merck, Germany), and pressed into a tablet form, and the spectrum was recorded with the relevant wave number interval of 4600-400 cm^{-1}.

The surface area of the poly(HEMA-MAGA) nanoparticles was calculated using the following Equation 1:

$$N = 6 \times 10^{10} \times S / \pi \times \rho_s \times d^3 \quad (1)$$

where, N is the number of nanoparticles per milliliter; S is the % of solids; ρ_s is the density of bulk polymer ($g.mL^{-1}$); d is the nanoparticle diameter (nm). The number of nanoparticles in mL suspension was determined by utilizing from mass-volume graph of nanoparticles. From all these data, specific surface area of the nanoparticles was calculated by multiplying N and surface area of 1 g nanoparticle.

Fe^{3+} removal studies from aqueous solutions

Fe^{3+} removal experiments were carried out batchwise in the media at different pH values. The poly(HEMA-MAGA) nanoparticles (10 mg) were added to adsorption medium (10mL) in suspended form, and the adsorption was performed in the flasks by stirring magnetically at 200 rpm for 2 h. Fe^{3+} solutions was prepared from its nitrate salt, ($Fe(NO_3)_3 \cdot 9H_2O$) by dissolving in deionized water with a conductivity value of 18.2 MΩ (supplied from Barnstead Nano pure Diamond). Effects of pH (i.e., 2-7 adjusted with HCl-NaOH) and initial concentration of Fe^{3+} (i.e., 0-100 $mg.L^{-1}$) on the adsorption rate and capacity were investigated. Due to the importance of fast removal of Fe^{3+} ions from GI tract in acute iron poisoning, adsorption time (15, 30, 45, 60, 90 and 120 min) was also studied. Removal efficiency was determined from the difference between initial and final Fe^{3+} concentraion in medium by using ICP-OES.

The amount of removed Fe^{3+} ions per unit mass of the nanoparticles was evaluated by using the mass balance. The removal capacity of the poly(HEMA-MAGA) nanoparticles for Fe^{3+} was calculated according to the following equation (Equation 2):

$$Q = [(C_i - C_f)V]/m \quad (2)$$

where, Q is the amount of Fe^{3+} ions adsorbed onto unit mass of the nanoparticle ($mg.g^{-1}$); C_i and C_f are concentration of Fe^{3+} ions in the initial and in the final solution after treatment, respectively ($mg.mL^{-1}$); V is the total volume of the solution (L), and m is the mass of the nanoparticles (g).

Fe^{3+} removal studies from intestinal mimicking solutions

Preparation of Fe^{3+} ions contain intestinal mimicking medium (A): For the batchwise adsorption tests, intestinal mimiking solution was prepared [40]. A total of 200 mL water was added to 125 mL of a 0.2 M potassium dihydrogen phosphate solution and 95 mL of 0.2 M sodium hydroxide solution. Then, 24.5 g of sodium deoxycholate (NaDC) and 16.5 g sodium cholate (NaC) were added. The pH was adjusted to 4.0±0.1 with 0.2 M sodium hydroxide solution and final volume was made up to 500 mL using water. After sparging with nitrogen for 30 min, the solution was stored in dark at room temperature.

Preparation of iron standard solution (B): To 500 mL of (A) solution above, 900 mg of iron sulfate ($FeSO_4 \cdot 7H_2O$) (2.7801 g, 0.10 mol) was added, and the solution was stirred for 2 h at 50°C. The solution was then sparged with nitrogen for 30 min and stored in dark at room temperature.

Binding Experiments: A total of 10 mg of dry nanoparticles was suspended into 20 mL of intestinal mimicking solution (IMS). The samples were then magnetically stirred for 1 h, at a stirring rate of 100 rpm. The solution was centrifuged to separate the nanoparticles, and the supernatant solution was estimated for iron. The amount of removed Fe^{3+} was calculated by using a calibration curve obtained from the same experiments.

Selectivity studies

A solution (20 mL) containing 10 $mg.L^{-1}$ of each metal ion was treated with the Fe^{3+} imprinted nanoparticles at a pH 4.0 in the flasks stirred magnetically at 200 rpm. The selectivity of MIP particles for Fe^{3+} was investigated by using Cd^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+} as interfering ions since their molecular radius are quite similar with iron. The selectivity studies were performed in intestinal mimicking solution including 10 mg of nanoparticles was weighed and added 20 mL of above solution containing competitive metal ions in a flask. Flask was magnetically stirred in for 2h at a stirring rate 200 rpm. The polymer suspension was centrifuged (20.000 rpm for 30 min) and concentration of metal ions in the supernatant was determined by ICP-OES. The amount of metal ions bound to the polymer was calculated by difference.

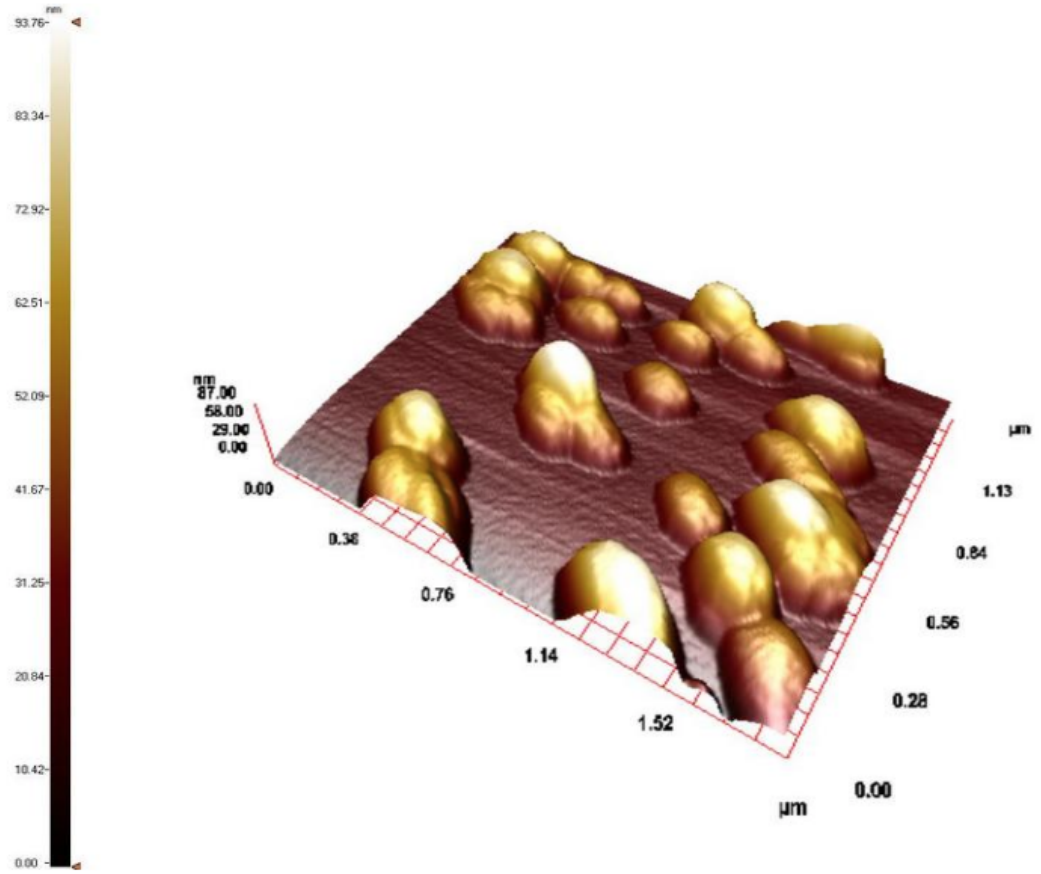

Figure 2. AFM photograph of the poly(HEMA-MAGA) nanoparticles.

Distribution and selectivity coefficients (K_d) of competitive ions with respect to Fe^{3+} ions were calculated by the following Equation. (3)

$$K_d = [(C_i - C_f)/C_f] \times V/m \quad (3)$$

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

The selectivity coefficient (k) for the binding of Fe^{3+} in the presence of other competitive metal ions (Equation. 4) can be obtained from equilibrium binding data according to (Equation. 4).

$$k = K_d \text{ (iron)} / K_d \text{ (comp.)} \quad (4)$$

Where, k is the selectivity coefficient, and k represents competitive metal ions.

A comparison of the k values of the MIP nanoparticles allows an estimation of the effect of imprinting on selectivity. The relative selectivity coefficient (***k´***) is an indicator for competitive binding efficiency of imprinted nanoparticles recognition sites in terms of non imprinted nanoparticles.

A relative selectivity coefficient k' (Equation. 5) can be defined as

$$k' = k_{MINps} / k_{NINps} \quad (5)$$

RESULTS and DISCUSSION

Characterization of Nanoparticles

Size and spherical shape of the nanoparticles provide them high surface area due to this unique feature of nanoparticles used as suitable adsorbent for the adsorption process. The specific surface area of poly(HEMA-MAGA) nanoparticles was calculated as 895 $m^2.g^{-1}$ by using Equation 1. Figure 3. shows the average size and size dist-

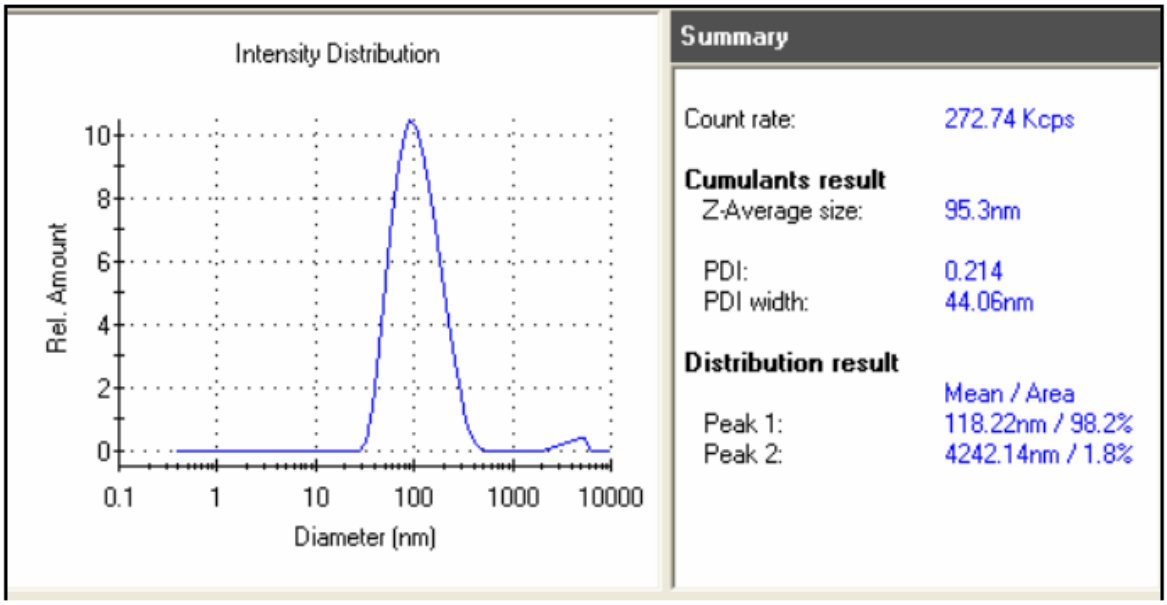

Figure 3. Particle size and size distribution of poly(HEMA-MAGA) nanoparticles.

ribution of poly(HEMA-MAGA) nanoparticles. The average size and size distribution of nanoarticles in suspension form were obtained by zeta sizer as about 95.3 nm with a polydispersity index (PDI) of 0.214.

AFM was also used to evaluate the morphology and size distribution of the prepared nanoparticles. Figure 2. shows AFM image of poly(HEMA-MAGA) nanoparticles. The average size and spherical morphology of Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was determined and confirmed by AFM. AFM images shows that the poly(HEMA-MAGA) nanoparticles are spherical and uniform with maximum size recorded as 93.7 nm. The average sizes of Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were recorded by zeta size analyzer as 95.3 nm, which was in conformity with AFM observations.

The chemical structure and functional groups content of the poly(HEMA-MAGA) nanoparticles were confirmed by FTIR analysis. It can be clearly seen that characteristic stretching vibration bands for the poly(HEMA-MAGA) nanoparticles were observed in FTIR spectrum in Figure 4.

The broad peak observed near 3442 cm^{-1} was assigned to -OH stretching vibrations while the characteristic strong stretching vibration band at 1729 cm^{-1} was attributed to carbonyl groups. As it is seen in FTIR spectrum, due to the proportion of functional MAGA monomer in poly(HEMA-MAGA) nanoparticles, carbonyl band was appeared quite intensive. Characteristic stretching vibration band amide I and amide II absorption bands were observed at 1637 cm^{-1} and 1454 cm^{-1}, respectively as in Figure 4.

Fe^{3+} Removal Experiments from Aqueous Solutions

Effect pH on Fe^{3+} removal efficiency

Since pH plays a important role in metal-chelate formation, the effect of pH on the Fe^{3+} removal efficiency of poly(HEMA-MAGA) nanoparticles was investigated. Experiments were carried out at different pH values of iron solutions ranging from 2.0 to 7.0. The pH dependence of Fe^{3+} adsorption onto Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was shown in Figure 5. As clearly seen here, maximum Fe^{3+} adsorption was achived at pH 4.0. Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles exhibited high affinity to Fe^{3+} ions in acidic medium than basic one. This means that the carboxyl groups of the MAGA monomer on the surface of the nanoparticles deprotonated to carboxylate form in acidic pH. Hereby, electorostatic interaction between nanoparticles and Fe^{3+} ions was enhanced. Fe^{3+} adsorption at pH 4.0 with an initial concentration of 5 $mg.L^{-1}$ was reached to maximum value of 23.6 $mg.g^{-1}$. This can be explained by the fact that, at this pH, most of pendant carboxyl groups are protonated. This results indicate that Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles is suitable for Fe^{3+} removal at gastric pH.

Significantly lower removal capacities were obtained with in more acidic and in more alkaline pH regions. The decrease in the Fe^{3+} removal capacity in more aci-

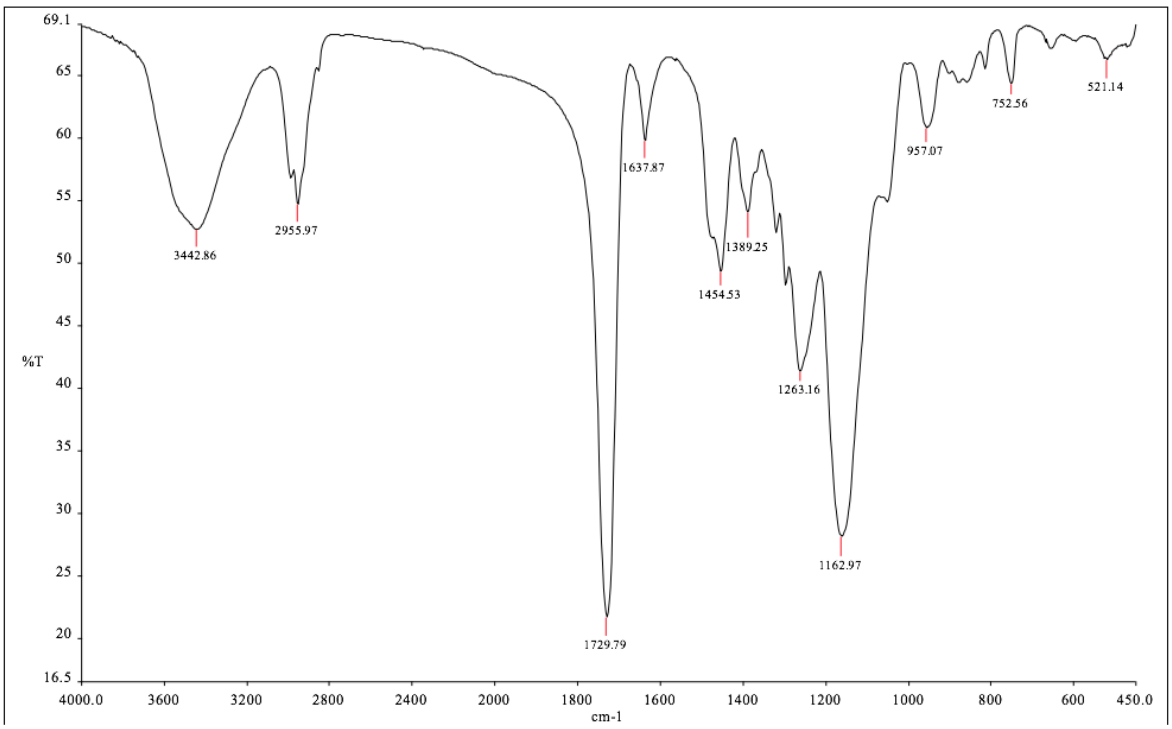

Figure 4. FTIR spectrum of poly(HEMA-MAGA) nanoparticles.

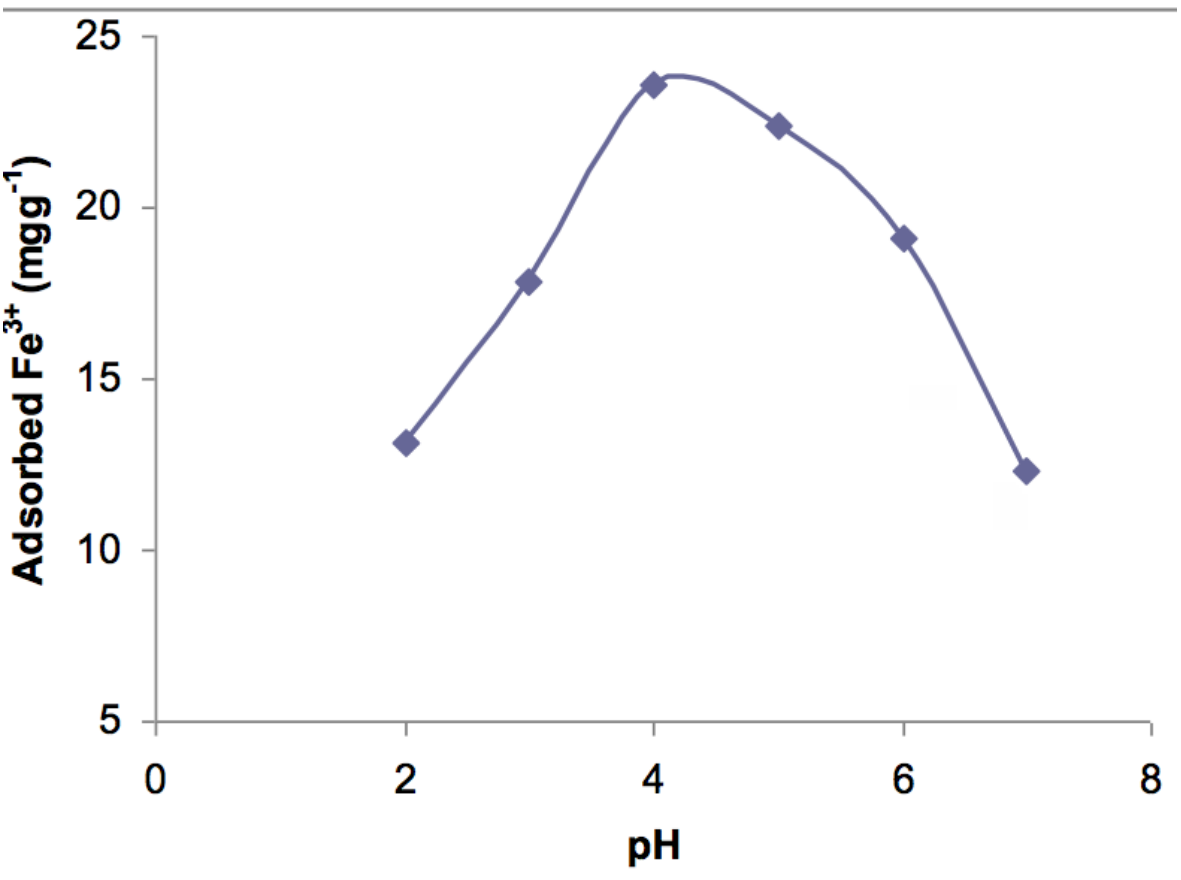

Figure 5. Effect of pH on Fe^{3+} removal capacity of poly(HEMA-MAGA) nanoparticles. İnitial concentration of Fe^{3+}: 5 $mg.L^{-1}$; T: 25°C.

dic and more alkaline pH regions can be attributed to electrostatic repulsion effects between Fe^{3+} ions and charged carboxyl.

Effect of Contact Time on Fe^{3+} removal efficiency

All acute poisoning time is very important for treatment, because the poison should be removed before acces of Fe^{3+} ions to blood circulation system. After swallow of the toxic substans like iron, oral chelator should form insoluble and nontoxic complex in intestinal system through the faecal excretion routes. Figure 6 shows time dependent adsorption of the Fe^{3+} ions on to the poly(HEMA-MAGA) polymeric nanochelators. As seen in figure, iron adsorption reached equilibrium in 60 minutes, and mostly Fe^{3+} ions removed in 20 minutes. Due to the large surface area of the poly(HEMA-MAGA) nanoparticles, Fe^{3+} ions were removed very rapidly when comparing with conventional adsorbents.

Effect of Fe^{3+} ions concentration in solution on Fe^{3+} removal efficiency was investigated and the results were shown in Figure 7. The amount of Fe^{3+} ions bound onto the poly(HEMA-MAGA) nanoparticles increased with increasing Fe^{3+} concentration and then reached a saturation value (i.e., 204,8 $mg.g^{-1}$) at ion concentraiton of 50$mg.L^{-1}$, which represents saturation of the active binding ionic cavities on Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles. It becomes constant when Fe^{3+} concentration is greater than 50 $mg.L^{-1}$. The maximum adsorption capacity was found to be 204.8 $mg.g^{-1}$ dry weight of poly(HEMA-MAGA) nanoparticles. This value means that only 1 gr polymeric chelater can remove 60 mg Fe^{3+} contains 4 iron tablets from the medium.

Results of selectivity experiments

Selectivity is an important parameter for MIP chelating agent. In order to determine Fe^{3+} ion recognition capability of ion-imprinted poly(HEMA-MAGA) nanoparticles, selectivity experiments carried out in the presence of competitive ions such as Cd^{2+}, Co^{2+}, Cr^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+} in a batch system. These ions were chosen as competitive metal ions because of their similar ionic radii or ionic charges. Although these ions have similar chemical property, the competitive adsorption capacity of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles for Fe^{3+} ions is higher than competitive ions. Table 1. summarizes K_d, and k, values of competitive ions with respect to Fe^{3+} ions.

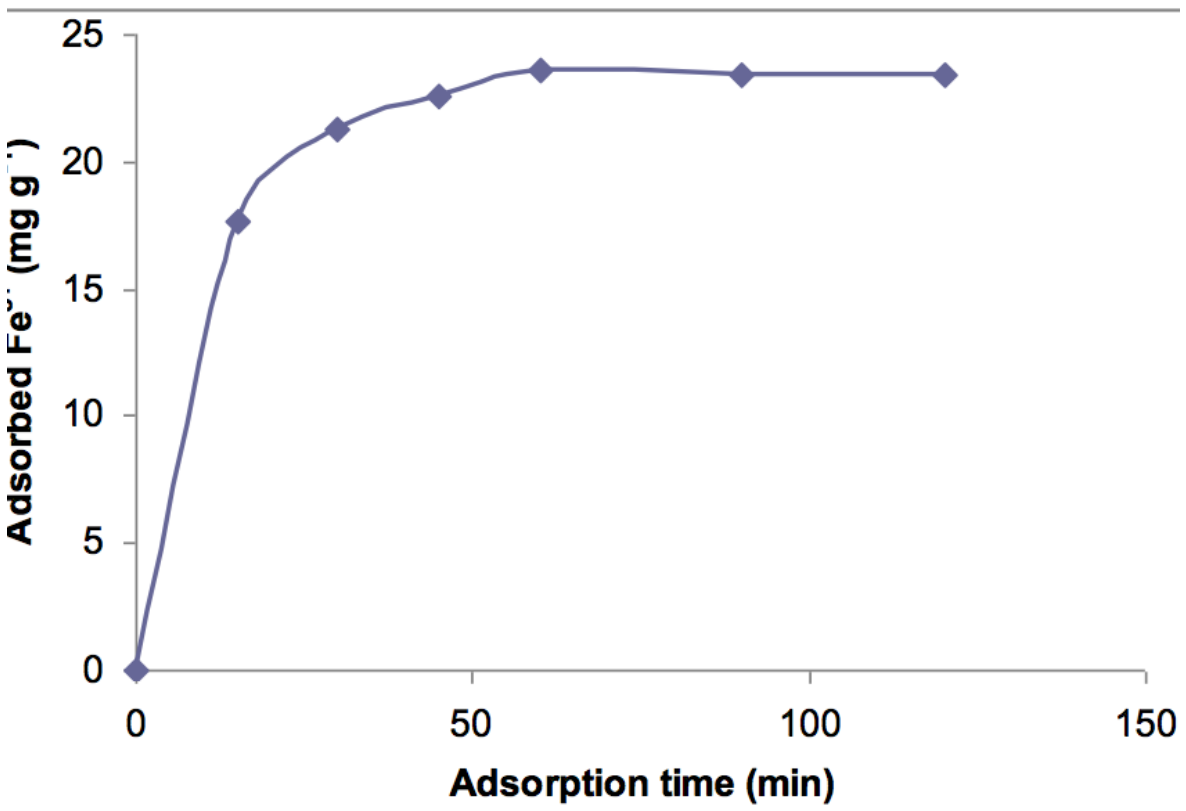

Figure 6. Effect of incubation time on Fe^{3+} removal capacity of the poly(HEMA-MAGA) nanoparticles: pH: 4.0; Initial concentration of Fe^{3+}: 5 $mg.L^{-1}$; T: 25°C.

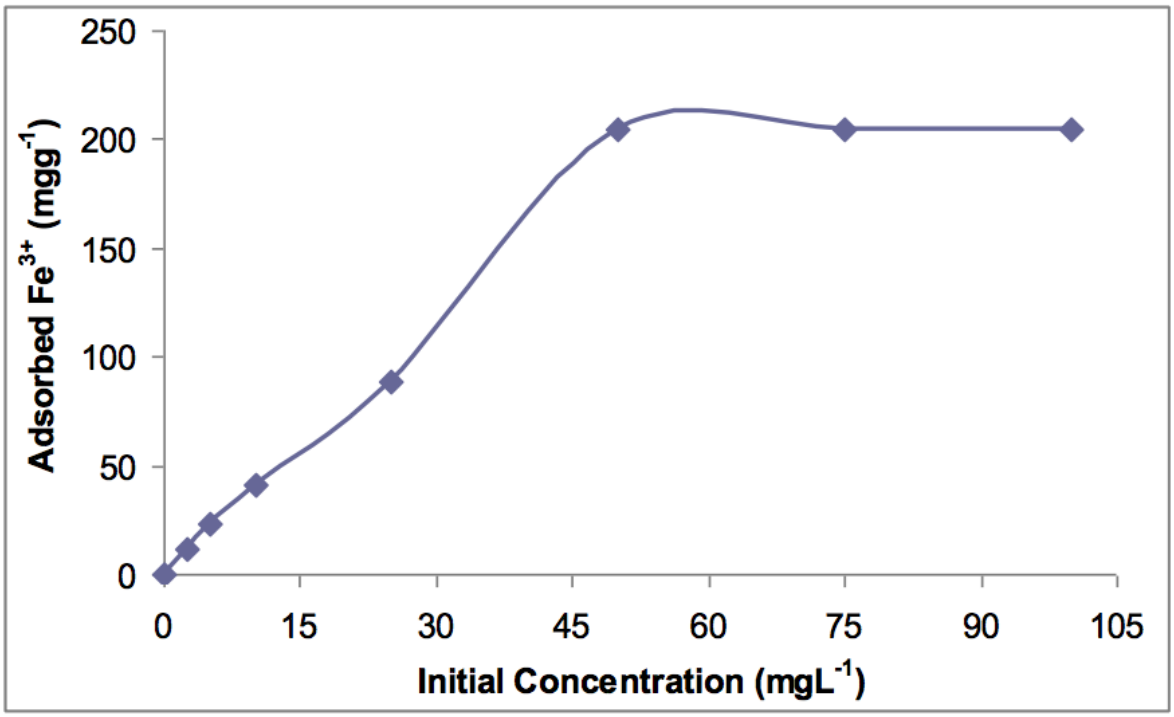

Figure 7. Effect of pH on Fe^{3+} removal capacity of poly(HEMA-MAGA) nanoparticles. İnitial concentration of Fe^{3+}: 5 mg.L^{-1}; T: 25°C.

The distribution coefficients (K_d), selectivity coefficients (k) and the relative selectivity coefficients (k') of ions were calculated as expressed above. The results of selectivity coefficients for each ion were listed in Table 1. The distribution coefficient (K_d) of Fe^{3+} ions for Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was significantly greater than the competitive ions.

The relative selectivity coefficient (k') defines the imprinting effect for Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles with respect to non-imprinted poly(HEMA-MAGA) nanoparticles. Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles were possess Fe^{3+} ion recognition cavities on the surface, the selectivity coefficients values (k') of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles for Fe^{3+}/Cd^{2+} Fe^{3+}/Co^{2+}, Fe^{3+}/Cr^{3+}, Fe^{3+}/Ni^{2+}, Fe^{3+}/Pb^{2+}, Fe^{3+}/Zn^{2+} and Fe^{3+}/Mn^{2+} almost were 196.5, 241.2, 218.3,191.5, 164.7, 147.2 and 252.8 fold higher than the non-imprinted poly(HEMA-MAGA) nanoparticles, respectively. The high selectivity of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles is due to the well designed coordination geometry of incorporated MAGA

Table 1. K_d, k, and k´ values of competitive ions with respect to iron.

Metal Ion	Non-imprinted NPs		Imprinted NPs		k'
	Kd (mL g^{-1})	k	Kd(mL g^{-1})	k	
Fe^{3+}	648.2	-	39388.5	-	-
Cd^{2+}	573.1	1.1	182.2	216.2	196.5
Co^{2+}	522.6	1.2	136.1	289.4	241.2
Cr^{3+}	696.4	0.9	200.4	196.5	218.3
Ni^{2+}	487.3	1.3	158.2	249.0	191.5
Pb^{2+}	562.5	1.2	199.3	197.6	164.7
Zn^{2+}	632.7	1.0	267.5	147.2	147.2
Mn^{2+}	518.6	1.2	129.8	303.4	252.8

molecules and Fe^{3+} ions. Selectivity results indicated that through ion-imprinting process, polymeric nanoparticles gained high selectivity for template Fe^{3+} ions due to the formed selective cavities.

Adsorption of Fe^{3+} from intestinal mimicking solution

In order to understand the in vivo performance of Fe^{3+} removal capacity of Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles, biorelevant media was used. Biorelevant media content was closely mimic the intestinal fluid of small intestine. An ionic strength and pH corresponding to that found in intestine was adjusted by addition of sodium hydroxide and potassium dihydrogen phosphate. To mimic in vivo conditions, experiments were carried out at 37°C, pH; 4, and stirred magnetically at 200 rpm for 2 h.

Testing of Fe^{3+} removal performance in intestinal mimicking with Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was provide us accurate prediction of behavier of chelating agent. After 30 minutes of the additon of the nanoparticles, the nanoparticle was removed from the medium and Fe^{3+} ion consantration of the medium was determined by IOCP. The amount of Fe^{3+} removed from the medium was calculated by subtracting the amount of Fe^{3+} ions before and after treatment with Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles. Time dependent Fe^{3+} removal performance of the polymer was showed in Figure 8.

The amount of the removed Fe^{3+} ions from intestinal mimicking medium by Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was higher than aqueous medium. 90% of Fe^{3+} ions removal from the intestinal mimicking medium was achived almost in 20 minutes by Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles. Fe^{3+} ions removal capacity of the Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles from medium was 206.4 $mg.g^{-1}$ polymer. These results were higher than Fe^{3+} removal capacity from aqueous solutions. Increasing of the removal capacity may be explained by the revealing the active functional groups of the polymer in the biorelevant solutions. So chemical chelations was increased with increasing the number of the revealed active sites.

Conclusion

Although acute iron poisoning is most common problem for children under age 6 years, there is no more orally active chelating agent. In this study, development of a polymeric Fe^{3+} chelating alternative agent was aimed. Due to the shortcoming of the clinically used chelating agents in effectiveness at GI tract and short plasma life we prepared

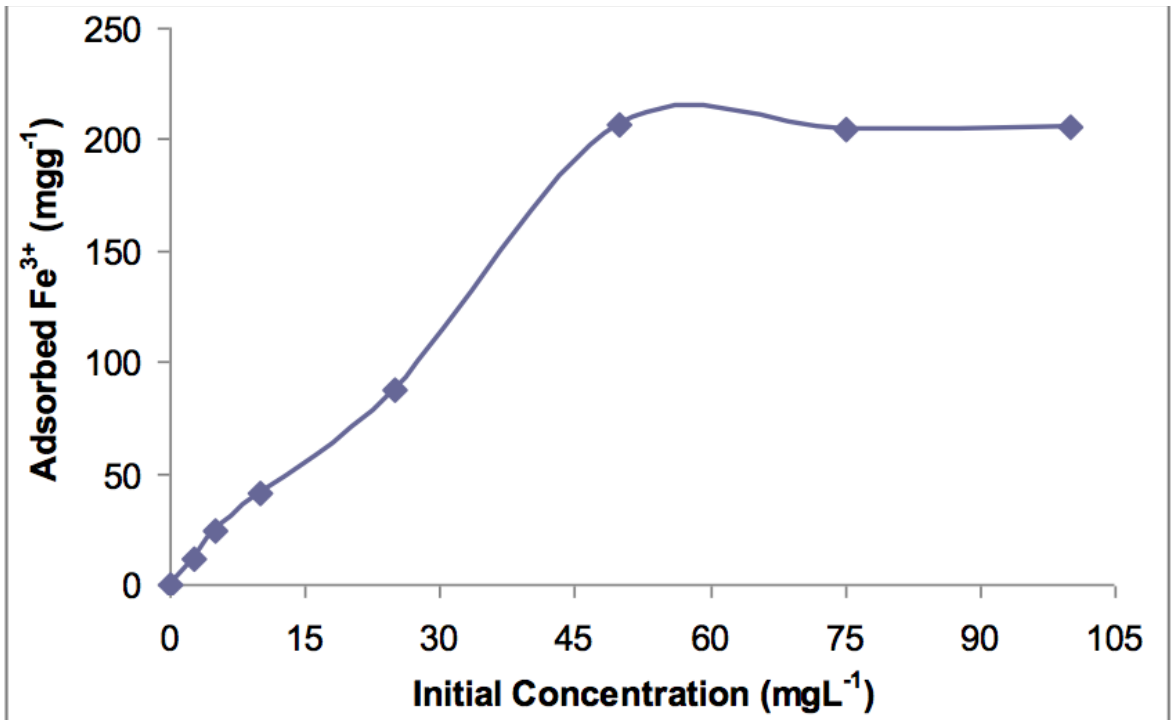

Figure 8. Fe^{3+} removal performance of the poly(HEMA-MAGA) nanoparticles in intestinal mimicking medium: pH: 4.0; T: 37°C.

acrylate based nanosized polymer, which contains glutamic acid as iron chelating agent. The iron selectivity of the polymer was enhanced by molecular imprinting techniques. The size and shape of the polymer were provided rapid removal capability and high adsorption capacity.

Ergün et al. [21] prepared Fe^{3+} imprinted poly(GMA-MAC) (MIP) beads embedded PHEMA composite cryogel for selective removal of Fe^{3+} ions from b-thalassemia patient plasma. The maximum adsorption amount of Fe^{3+} ions was 2.23 $mg.g^{-1}$. composite cryogels. Polomoscanik et al. [23] prepared Hydroxamic Acid-Containing hydrogel for Fe^{3+} chelation. poly(2-hydroxyethyl)acrylate (HEA) based hydrogel have been synthesized as potential nonabsorbed chelators for iron in the gastrointestinal tract. The maximum adsorption capacity was 81 $mmol.g^{-1}$ hydrogels. Zhou et al. [24] synthesized 3-hydroxypyridin-4-one hexadentate ligand-containing copolymers for to reduce the efficiency of iron absorption from the intestine by administering iron chelators. 3-Hydroxypyridin-4-one Hexadentate ligand synthesized and incorporated into polymers by copolymerisation with N,Ndimethylacrylamide (DMAA), and N,N'-ethylene-bis-acrylamide (EBAA). Their Fe^{3+} binding capacity was 271 $\mu mol.g^{-1}$ polymers. Yavuz et al. [31] investigated Fe^{3+} removal performance of Fe^{3+} imprinted poly(HEMA-MAGA) beads and poly(HEMA-MAGA) membranes [32] from iron overdosed human plasma. Their adsorption capacity was 92.6 $\mu mol.g^{-1}$ for beads and 164.2 $\mu mol.g^{-1}$ for membranes. In this study Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles was synthesized for iron removal in the GI tract and reached about 206,4 $mg.g^{-1}$ Fe^{3+} adsorption capacity in IMS.

Several methods were generated for elimination of excessive iron ions from body but, most of them aimed to remove of iron from tissues originated blood transfusion based disease. Many research groups have focused to developt novel chelating agent for oral treatment. Among these approaches, polymeric chelating agents have become attractive for researchers by their low cost, ready to preparation, non toxic effects and usefull in harsh condition like stomac solutions. In the light of the above discussion, we believe that Fe^{3+} imprinted poly(HEMA-MAGA) nanoparticles offeres the promising strategy with high removal capacity with specificity and efficiency of Fe^{3+} ions.

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