Therapeutic Affinity Adsorption of Iron(III) with Dye- and Ferritin-Immobilized pHEMA Adsorbent

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ABSTRACT: Microporous poly(2-hydroxyethylmethacrylate) (pHEMA) films carrying cibacron blue F3GA, Congo red, and ferritin were prepared and used for iron(III) removal from human plasma. pHEMA films were produced by a photopolymerization of 2-hydroxyethylmethacrylate in the presence of azobisisobutyronitrile. The reactive dye ligands cibacron blue F3GA, Congo red, and bioligand ferritin were then covalently attached to the pHEMA films. The maximum dye loadings were 1.07 and 0.80 μmol/cm^2 for cibacron blue and Congo red, respectively. The maximum amount of ferritin attached was 1.04×10^{-3} μmol/cm^2. Characterizations of the films were achieved by contact-angle, water-uptake, and scanning electron microscopy studies as well as atomic force microscopy images. The aqueous water-uptake properties and contact angles (air underwater) of the pHEMA films did not change after derivatization with cibacron blue F3GA, Congo red, and ferritin. These hydrophilic films (contact angle = 45.3°), having a swelling ratio of 58% (w/w) and carrying cibacron blue F3GA, Congo red, and ferritin, were used in Fe(III) removal studies. The maximum amounts of Fe(III) removed from human plasma by cibacron blue F3GA-, Congo red-, and ferritin-attached pHEMA films were 3.80 μg/cm^2 for cibacron blue F3GA, 4.41 μg/cm^2 for Congo red, and 8.1 μg/cm^2 for ferritin-attached films. Fe(III) ions could be repeatedly adsorbed and desorbed with these affinity pHEMA films without a noticeable loss in their Fe(III) adsorption capacity. J Appl Polym Sci 82: 186–194, 2001

Key words: pHEMA; affinity adsorbent; iron(III) removal; adsorption

INTRODUCTION

Iron is an essential trace element for almost all organisms.[1] The toxic effects of iron overload are well known, especially because the human body has no physiological route for the elimination of excess iron.[2] Chronic iron overload can be caused by a genetic defect, certain types of anemia, accidental ingestion, repeated blood transfusions, inhalation of tobacco smoke or asbestos, or overmedication with iron supplements or iron pills prescribed by a physician.[3] In acute iron overload, the iron transport proteins are overwhelmed, and unbound toxic iron (in excess of the total iron-binding capacity) is created, generating highly reactive free oxygen radicals causing lipid peroxidation and cell membrane damage. In chronic iron overload, a small nonspecific iron pool exists, but most of the iron is deposited in the organs, especially in the spleen, liver, and heart, causing widespread organ damage.[4]

For transfusional iron overload and acute iron poisoning, the only available supportive treatment is chelation therapy, and the only available

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clinical drug for this treatment is desferrioxamine B (DFO), a linear hydroxamate and a natural siderophore.[5] The use of DFO has already been shown to result in prolonged life expectancy, reduced liver iron, and the establishment of a negative iron balance. However, major limitations to the usefulness of DFO include its lack of effectiveness when administered orally, the short half-life time in plasma, and its potential toxicity in high concentrations.[6] DFO is very expensive also. For this reason, a number of orally active iron chelators are being tested, but none of them are still satisfactory.[7–9] To overcome the drawbacks of soluble iron chelators in the treatment of iron overload, researchers have studied the attachment of iron-chelating ligands. Compared with soluble iron chelators, iron-chelating resins might have advantages in stability, reusability, and minimal damage to biological substances.

Recently, we prepared poly(2-hydroxyethylmethacrylate) (pHEMA)-based microbeads carrying different bioligands (protein A, DNA, low molecular weight heparin, cibacron blue F3GA, and thionein) for the removal of toxic or undesired substances [e.g., pathogenic anti-DNA antibodies, cholesterol, bilirubin, and Cd(II) ions] from human plasma.[10–14] In this study, we aimed to prepare metal-chelate-forming microporous pHEMA films because of the many advantages of such film systems, including their high porosity, large internal surface area, and high chemical, biological, and mechanical stability. Cibacron blue F3GA, Congo red, and ferritin were covalently attached to the pHEMA films. These dye- and protein-modified films were used for *in vitro* Fe(III) removal from human plasma overloaded with Fe(III). This article presents data on the iron adsorption capacities and rates of these films carrying dyes and ferritin.

EXPERIMENTAL

Materials

2-Hydroxyethylmethacrylate (HEMA) was obtained from Sigma Chemical Co. (St. Louis, MO), distilled under reduced pressure in the presence of hydroquinone, and stored at 4°C until use. α,α'-Azobisisobutyronitrile (AIBN) was purchased from Fluka AG (Buchs, Switzerland) and used as received. Cibacron blue F3GA, cyanamide (carbodiimide), and ferritin (type 1, from horse spleen) were purchased from Sigma Chemical Co. and used as received. Congo red was supplied from BDH (Poole, UK) and also used as received. Coomassie blue for the Bradford protein assay was supplied from BioRad (Richmond, CA). All water used in the experiments was purified with 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. The resulting deionized water has a specific conductivity of 18 $M\Omega\ cm^{-1}$. Other chemicals used in this study were either laboratory-grade or analytical-grade and were purchased from Merck AG (Darmstadt, Germany).

Preparation of pHEMA Films

The pHEMA film was prepared by a UV photopolymerization technique according to Arıca et al.[15] HEMA was purchased from Sigma and was purified by vacuum distillation under a nitrogen atmosphere. The film preparation mixture (5 mL) contained 2 mL of HEMA, 5 mg of AIBN as a polymerization initiator, and 3 mL of 0.1*M* $SnCl_4$ as a pore former. The monomer mixture was then poured into a round glass mold (ϕ = 4.5 cm) and exposed to UV radiation for 10 min, and a nitrogen atmosphere was maintained in the mold. The film was washed several times with distilled water.

After polymerization, the pHEMA films were separated from the polymerization medium and cut into small circular pieces (1.0 cm in diameter and 600 μm thick) with a perforator. The residual chemicals (e.g., unconverted monomer, initiator, and other ingredients) were removed with a cleaning procedure described in detail elsewhere.[16] Briefly, film pieces were transferred into a reservoir, and washing solutions (i.e., a dilute HCl solution and a water–ethanol mixture) were recirculated through the system, which included an activated carbon column, until the films were clean. We followed the purity of the films by observing the changes in the optical densities of the samples taken from the liquid phase in the recirculation system and also from the DSC thermograms of the films obtained with a differential scanning microcalorimeter (Mettler, Switzerland). The optical density of the uncleaned film was 2.5. After the cleaning operation, this value was reduced to 0.04. In addition, when the thermogram of uncleaned film was recorded, it had a peak around 60°C. This peak might have originated from AIBN. However, after the application of this cleaning procedure, no peak was observed on this thermogram between 30 and 100°C.

Dye Attachment to pHEMA Films

Cibacron blue F3GA and Congo red were bound covalently to the pHEMA films. Briefly, 100 mL of an aqueous dye solution containing 4.0 g of NaOH was mixed with pHEMA film pieces. This solution was stirred magnetically at 400 rpm in a sealed reactor. The dye attachment reaction was carried out at 80°C for 4 h. A nucleophilic substitution reaction took place between the chloride of the cibacron blue triazine ring and the hydroxyl group of the HEMA, with the elimination of NaCl, resulting in the coupling of cibacron blue F3GA to the pHEMA films under alkaline conditions. Congo red was immobilized to the pHEMA films via the condensation reaction between the amine groups of the dye and the hydroxyl groups of the films under alkaline conditions. The initial concentration of the dye in the medium was 3.0 mg/mL. After dye attachment, the solution was cooled down to room temperature, and the dye-attached films were first filtered and then washed with distilled water. The modified films were further shaken with methanol overnight at room temperature. Then, modified films were washed again with water for removal of all the dye molecules physically attached on the surface and/or diffused into the pores. The dye-attached films were stored at 4°C with 0.02% sodium azide to prevent microbial contamination prior to use.

Dye Release Studies

To estimate the amount of released cibacron blue F3GA and Congo red, we placed the film samples in test tubes containing 10 mL of release media and shook them on a rotary shaker for 24 h. The media were replaced with fresh media every 24 h, and the experiment was continued until no measurable release was observed. The amount of dye released into the medium was measured cumulatively as the absorption bands at 630 nm for cibacron blue F3GA and at 497 nm for Congo red with a bench-top spectrophotometer (Spectronic-21 Series, Bousch and Lomb, Germany). Three kinds of release media were used: a pH 2.0 buffer of an acetic acid solution (50% v/v), a phosphate buffer solution (pH 7.0), and a sodium citrate/NaOH buffer solution (pH 12.0).

Polymer Characterization

Water Content of pHEMA Films

The water-uptake behavior of pHEMA films was determined in distilled water. Dry film pieces were placed in distilled water and kept at a constant temperature of 25 ± 0.5°C. These films were periodically removed and weighed with an electronic balance (Shimadzu, Japan, EB.280 ± 1.10^{-4} g). The water content of the films was calculated with the following expression:

$$\text{Water uptake ratio } \% = [(W_s - W_o)/W_o] \times 100 \quad (1)$$

where W_o and W_s are the weights of the films before and after water uptake, respectively.

Contact-Angle Measurements

The plain, dye-attached, and ferritin-attached pHEMA films were characterized with an air-under-water contact-angle measuring technique.[17] The device consisted of a travelling goniometer with 15× eyepieces, a variable intensity light source, and a micrometer-adjustable X–Y stage vertically mounted on an optical bench. The stage contained a Plexiglas container in which a Teflon plate was suspended. A polymer sample was held on the underside of the Teflon plate with small Teflon clips. The container was then filled with triple-distilled water, and the plate with the sample was lowered into the container until the sample was completely immersed. A bubble of air with a volume of about 0.5 μL was then formed at the tip of the Hamilton microsyringe below the surface, detached, and allowed to rise to the polymer–water interface. The air bubbles were photographed at 25°C within 5 min for reaching equilibrium after contact with the pHEMA samples. The equilibrium contact angle (θ_{air}) was calculated from the height (h) and width (b) of the air bubble at the pHEMA sample surface with the following equation. The mean value of five contact angles measured on bubbles at different positions was considered. The reproducibility of the contact angles was ±2%:

$$\theta_{air} = \cos^{-1}[(2h/b) - 1] \quad \text{for} \quad \theta_{air} < 90° \quad (2)$$

where θ_{air} is less than 90°.

Elemental Analysis

The amounts of cibacron blue F3GA and Congo red covalently attached to the pHEMA films were determined with an elemental analysis instrument (CHNS-932, Leco, Chicago, IL) by the

measurement of the nitrogen and sulfur stoichiometry.

Scanning Electron Microscopy (SEM)

SEM micrographs of the pHEMA film were obtained with a Leitz AMR-1000 (Frankfurt, Germany) after coating with gold under vacuum.

Atomic Force Microscopy (AFM) Studies

To observe the surface topography of the pHEMA films, we took AFM micrographs with an atomic force microscope (Topometrix TMX 2000 Explorer in contact mode in air, Sunnyvale, CA). The scanner and tip used with this microscope were a 130-μm tripod and a pyramidal type for topographic images, respectively; the force exerted by the probe on the surface was 0.7 n*N*.

Carbodiimide Activation of pHEMA Films

Prior to the activation process, pHEMA film pieces were kept in distilled water for about 24 h and washed on a glass filter with a 0.5*M* NaCl solution and water for the system to come to equilibrium. A carbodiimide aqueous solution (100 mL) with a constant initial concentration (5.0 mg/mL) was prepared in a phosphate buffer (pH 10.0). The pHEMA films were then added to this solution. The suspension was gently agitated at room temperature (25°C), and the activation procedure was continued for 24 h at a constant pH of 10.0. After the activation reaction, to remove the excess activation agent, we filtered the pHEMA films, washed them with 2 $\times$ 100 mL of water, and continued washing them with 2 $\times$ 50 mL of acetone. NaCl was used to desorb the nonspecifically adsorbed species, and the film pieces were further washed with 2 $\times$ 25 mL of a 0.1*M* KH_2PO_4/K_2HPO_4 buffer (pH 7.5). Then, the modified films were stored at 4°C prior to use.

Ferritin Attachment

Carbodiimide-activated pHEMA films were magnetically stirred (at 50 rpm) at a constant temperature of 25°C for about 24 h (i.e., equilibrium time) with 10 mL of a ferritin solution. The total external surface area of the dry pHEMA films used in each batch was 100 cm^2/L. To observe the effect of the pH on the covalent coupling of ferritin to the carbodiimide-activated pHEMA films, the medium pHs were varied between 4.0 and 8.0. The initial concentration of ferritin was 0.5 mg/mL. To obtain the effect of the ferritin concentration on coupling, we varied the initial concentration of ferritin between 0.05 and 2.00 mg/mL, in which the pH of the solution was 4.0. After coupling, the ferritin-attached pHEMA films were washed with water and 0.01*M* ethylene diamine tetraacetic acid (EDTA) (pH 4.9). We determined the amount of ferritin attached on the carbodiimide-activated pHEMA films by measuring the decrease of ferritin concentration and also by considering the ferritin molecules adsorbed nonspecifically (the amount of ferritin adsorbed on the plain pHEMA films) by the Bradford method.

Fe(III) Removal from Human Plasma

Fe(III) removal from human plasma by cibacron blue F3GA-, Congo red-, and ferritin-carrying films was also studied batchwise. The blood samples (500 mL) were supplied from a healthy donor at the University Hospital (Hacettepe, Ankara). Blood samples were centrifuged at 500 g for 30 min at room temperature to separate plasma. Plasma (10 mL) was overloaded with 0.5 mL of an Fe(III) solution. Then, Fe(III)-overloaded human plasma was incubated with films at 25°C for 6 h (the total external surface area of the dry films used in each batch was 100 cm^2/L). The amounts of Fe(III) removed from human plasma [i.e., the plasma Fe(III) concentrations] were determined spectrophotometrically at 595 nm (FER-KIT, Bio Merieux, Marcy-l'Etoille, France) through the decrease in the Fe(III) concentration in plasma samples. The amount of adsorption per unit surface area of the films was calculated with the following expression:

$$Q = [(C_o - C).V]/A \tag{3}$$

where Q is the amount of Fe(III) ions adsorbed onto the unit surface area of the films ($\mu g/cm^2$); C_o and C are the initial and final concentrations of the Fe(III) ions in the plasma for a certain period of time, respectively (μg/dL); V is the volume of the plasma (dL); and A is the outer surface area of the pHEMA films used (cm^2).

Desorption and Repeated Use

The desorption of Fe(III) was studied in a 0.1*M* EDTA solution (pH 4.9). Fe(III)-loaded films were placed in this desorption medium and stirred (at a stirring rate of 600 rpm) for 2 h at room temperature. The total volume of the desorption medium

was 50 mL. The final Fe(III) concentration in the aqueous phase was determined with the same method described in the previous section. The desorption ratio was calculated from the amount of Fe(III) ions adsorbed on the films and the final concentration in the desorption medium, with the following equation:

$$\text{Desorption ratio (\%)} = \frac{\text{Amount of Fe(III) desorbed to the elution medium}}{\text{Amount of Fe(III) adsorbed on the films}}$$

To obtain the reusability of the dye- and ferritin-attached pHEMA films, we repeated the Fe(III) adsorption–desorption procedure three times with the same polymeric sorbent. After the desorption of Fe(III) ions with EDTA, possibly adsorbed protein molecules from human plasma (especially albumin through cibacron blue F3GA and Congo red molecules) were eluted with 0.1*M* KSCN (pH 8.0). Moreover, after the desorption of Fe(III) ions with EDTA, the dye and ferritin release was also monitored.

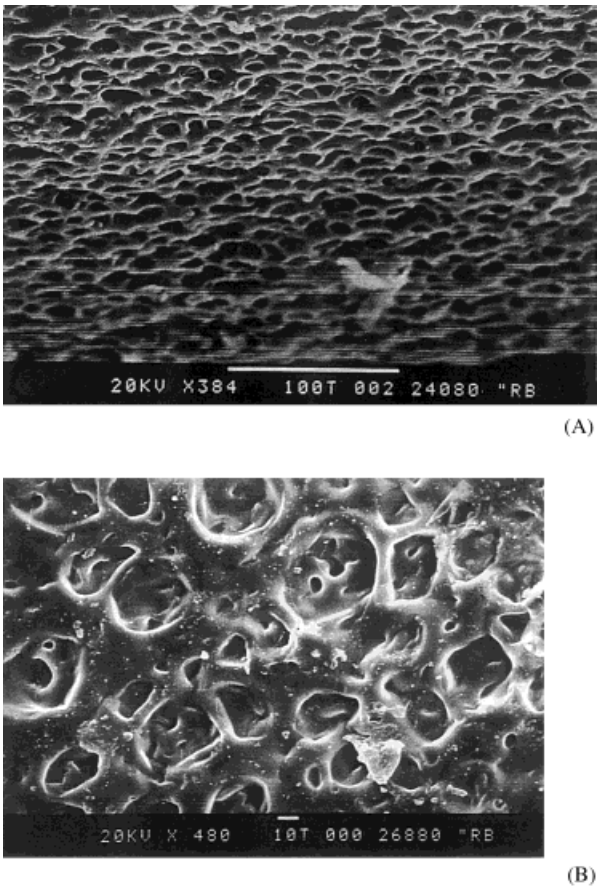

Figure 1 SEM micrographs of pHEMA film: (A) cross section and (B) surface.

RESULTS AND DISCUSSION

Polymer Characterization

Dye- and ferritin-attached microporous pHEMA films were prepared as a specific affinity sorbent for the removal of Fe(III) ions from human plasma. The main criteria for selecting pHEMA are its physiological acceptability, mechanical strength, chemical and biological stability, and good blood tolerance. From our observations, we have concluded that pHEMA is resistant to the adhesion of blood proteins (i.e., serum albumin, fibrinogen, and gammaglobulins) and blood cells (i.e., platelets and leukocytes).[18] The microporous pHEMA films prepared in this study were rather hydrophilic and crosslinked structures, that is, hydrogels. The simple incorporation of water weakens the secondary bonds within the hydrogels. This enlarges the distance between the polymer chains and causes the uptake of water. The equilibrium water-uptake ratio of the pHEMA films was 58% (w/w). The water-uptake properties of the pHEMA films did not change after carbodiimide activation and dye/ferritin attachment.

Characterization of the surface chemistry is a crucial issue in affinity technology. Contact-angle techniques are used routinely in the characterization of biomaterials to describe hydrophilicity (wettability) or to estimate surface energy. The contact angles were measured through the water phase, so relatively small contact-angle values indicate a relatively more hydrophilic surface. In other words, the contact angle represents a measurement of surface hydrophilicity (or wettability). There is a common opinion based on experimental evidence that blood compatibility can be increased by rendering biomaterials (e.g., films or microbeads) in the sense of containing more hydrophilic groups in their active surface and/or within the material matrix. The θ_{air} values were 45.3, 45.7, 45.5, and 45.9° for plain, cibacron blue F3GA-, Congo red-, and ferritin-modified films, respectively. As seen here, no further change in the contact angle was observed with the attachment of cibacron blue F3GA, Congo red, and ferritin molecules on the surface of pHEMA films.

SEM micrographs given in Figure 1(A,B) show the cross section and surface structures of

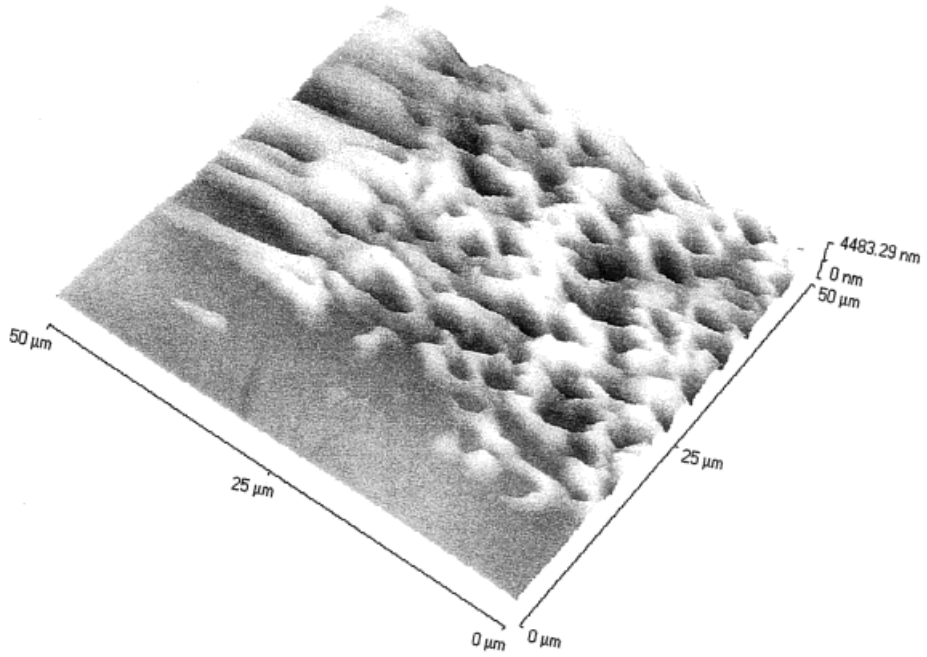

Figure 2 AFM image showing the surface morphology of pHEMA film. The AFM image was shaded by computer reprocessing to enhance the surface structure. The area of the image is 100 μm $\times$ 100 μm.

pHEMA film, respectively. As clearly seen, the film has large voids (transport canals); the micropore dimensions are greater than 1 μm. In Figure 1(B), the pHEMA surface seems very rough and heterogeneous. The large pores reduce diffusional resistance and facilitate mass transfer because of the high inner surface area. This also provides higher dye and ferritin attachment and enhances Fe(III) removal capacity. No significant changes in the film outer layer morphology were observed after dye and ferritin modification.

The topography of the pHEMA film surface was also examined by AFM. As seen in Figure 2, the pHEMA surface demonstrated high roughness and heterogeneity, as expected. The mean surface roughness (RMS) of unmodified pHEMA film was found to be 4483.3 nm imaged in contact mode. The mean surface roughness (RMS) did not change with dye and ferritin attachment.

Dye Attachment

Th dye ligands cibacron blue F3GA and Congo red were covalently attached to the pHEMA films under alkaline conditions and were used as the chelating agents for the specific removal of iron. As shown in Figure 3, cibacron blue F3GA contains three acidic sulfonate groups and four basic secondary amino groups; Congo red contains two acidic sulfonate groups and two secondary amino groups. The complexation of Fe(III) ions to the dye molecules occurs via these functional groups. It is accepted that ether linkages are formed between the reactive triazine ring of cibacron blue F3GA and the hydroxyl groups of pHEMA films. In the case of Congo red, covalent bonds are formed as a result of the condensation reactions

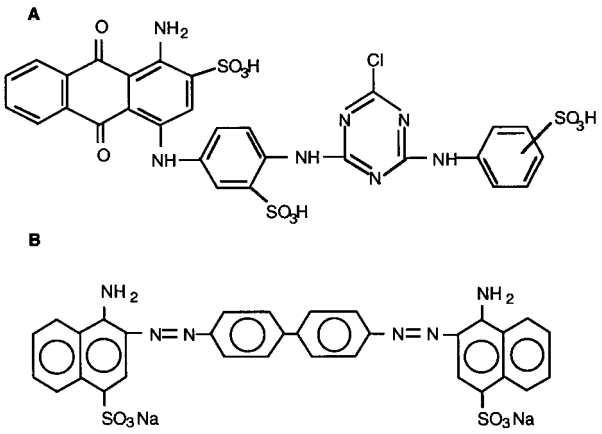

Figure 3 Chemical structures of the cibacron blue F3GA and Congo red molecules.

between the aromatic amine groups of the dyes and the hydroxyl groups of HEMA. Cibacron blue F3GA- and Congo red-attached pHEMA films were subjected to elemental analysis. The amounts of dye attached to the films were calculated from these data (on the basis of the stoichiometry) to be 1.07 and 0.80 μmol/cm^2 for cibacron blue F3GA and Congo red, respectively.

The release of dye molecules was measured in three different kinds of media. There was no measurable release of dye molecules into the acidic medium (pH 2.0). Dye was released in the neutral medium, and some was released in the alkaline medium as well. The release in the strongly alkaline medium indicates the existence of strong ionic interactions. The release in the neutral medium might just be the physically occluded dye molecules along with any weakly or physically bonded dye. There was not a significant increase in the amount of dye released.

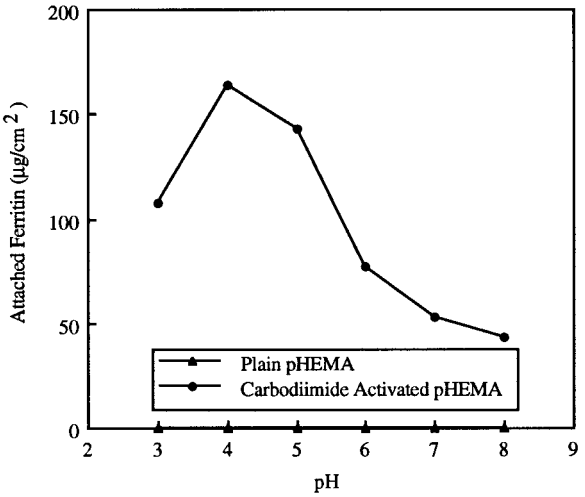

Figure 4 Effect of the incubation medium pH on ferritin attachment. The carbodiimide concentration in the activation medium was 5.0 mg/mL, the initial concentration of ferritin was 0.5 mg/mL, and the temperature was 25°C.

FERRITIN ATTACHMENT

The other Fe(III) complexing ligand chosen for this study is ferritin. Ferritin is a widely distributed iron-storage protein thought to be very important in providing protection against the catalysis of deleterious oxidation of biomolecules by iron.[19] It is an almost spherical major iron-storage plasma protein with a molecular mass of 440 kDa. It is composed of 24 equivalent subunits arranged in a shell-like manner with a hollow core with a diameter of 7.5 nm. Its outer shell diameter is 12.5 nm, as determined by transmission electron microscopy.[20] On the basis of these data, we have concluded that pHEMA film has effective pore structures for the attachment of ferritin. Ferritin contains approximately 2500 iron-binding (chelation) centers. This makes it an attractive chelating ligand for the removal of iron in iron poisoning.

Effect of pH

As shown in Figure 4, the effect of pH is significant. The maximum adsorption of ferritin (165 μg/cm^2) was observed at a pH of 4.0, which is the isoelectric pH of ferritin. The nonspecific adsorption of ferritin on plain pHEMA films was very low (5.0 μg/cm^2). Both the three-dimensional structure (conformational stability and more folded structure) and the ionization degree of several groups (i.e., amino acid side chains) on the ferritin molecules can change with pH. At pH 4.0, ferritin molecules probably have the most suitable structural properties for binding through carbodiimide-activated sites on the surface of pHEMA films. At pH values higher than 4.0, the attached amount of ferritin drastically decreases. This could be caused by the ionization state of ferritin caused by repulsive electrostatic forces between ferritin and active groups on the surface. An increase in conformational size and lateral electrostatic repulsions between adjacent adsorbed ferritin molecules may also cause a decrease in attachment efficiency.

Effect of Initial Ferritin Concentration

Figure 5 shows the nonspecific and specific adsorption onto the plain and carbodiimide-activated pHEMA films. One of the main requirements in bioaffinity chromatography is the specificity of the sorbent. The nonspecific interaction between the carrier matrix (here the pHEMA films) and the molecules to be adsorbed (here ferritin) should be minimal for a high specificity. As presented in this figure, with increasing ferritin concentration in solution, the amount of ferritin adsorbed per unit surface area by the carbodiimide-activated films increased. It became constant when the ferritin concentration was greater than 1.50 mg/mL. A negligible amount of ferritin was adsorbed nonspecifically on the plain

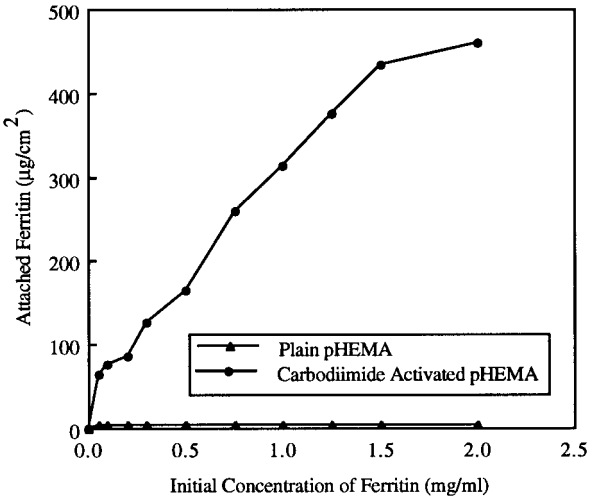

Figure 5 Effect of the ferritin concentration on ferritin attachment. The carbodiimide concentration in the activation medium was 5.0 mg/mL, the medium pH was 4.0, and the temperature was 25°C.

pHEMA films (5.0 μg/cm^2), whereas carbodiimide activation significantly increased the ferritin adsorption capacity of the films (up to 460 μg/cm^2). It is clear that this increase in the ferritin adsorption capacity is due to active binding sites on the carbodiimide-activated pHEMA films for ferritin attachment.

FE(III) REMOVAL FROM HUMAN PLASMA

Figure 6 gives removal rates of Fe(III) ions onto the films from human plasma. Desorption equilibrium was achieved in 5 h for all films studied. In chronic dialysis, an average treatment time is around 3–5 h. High mass-transfer rates are necessary to keep the treatment time to a minimum and meet therapeutic demands. This equilibrium removal period seems to be satisfactory for extracorporeal therapy for Fe(III) removal from human plasma. Fe(III) adsorption to ferritin-attached films was much faster than to dye-attached films. Fe(III) adsorption capacities were 3.8, 4.4, and 8.1 μg/cm^2 for cibacron blue F3GA-, Congo red-, and ferritin-attached films, respectively. About 60.5, 32.5, and 12.5% of the total Fe(III) in the human plasma were removed with the ferritin-, Congo red-, and cibacron blue F3GA-carrying pHEMA films, respectively. The total Fe(III) concentrations were reduced to about 350, 270, and 158 μg/dL in 6 h for cibacron blue F3GA, Congo red, and ferritin ligands, respectively. The affinity order of the adsorption rate was ferritin > Congo red > cibacron blue F3GA. In conclusion, it can be said that Fe(III) levels in blood plasma can be reduced significantly with relatively fast adsorption rates with the dye- and ferritin-modified pHEMA films developed in this study.

Desorption and Repeated Use

The desorption of Fe(III) ions from the pHEMA/cibacron blue F3GA, pHEMA/Congo red, and pHEMA/ferritin films was also studied in a batch experimental setup. The films loaded with Fe(III) ions were placed within the adsorption medium containing 25 m*M* EDTA at pH 4.9, and the amount of Fe(III) desorbed in 1 h was determined. The desorption ratio was calculated with eq. (4). Human plasma was used for repeated Fe(III) adsorption cycles.

Up to 99% of the adsorbed Fe(III) ions was desorbed. There was no dye and ferritin release in this case, which shows that ligand molecules were attached covalently to the pHEMA films. This means that EDTA breaks down only the chelates between Fe(III) ions and dye/ferritin molecules. In addition, when EDTA was used as a desorption agent, the coordination sphere was disrupted; subsequently, the ferritin changed conformation and released the bound Fe(III) ions. On the basis of the desorption data previously given, we have

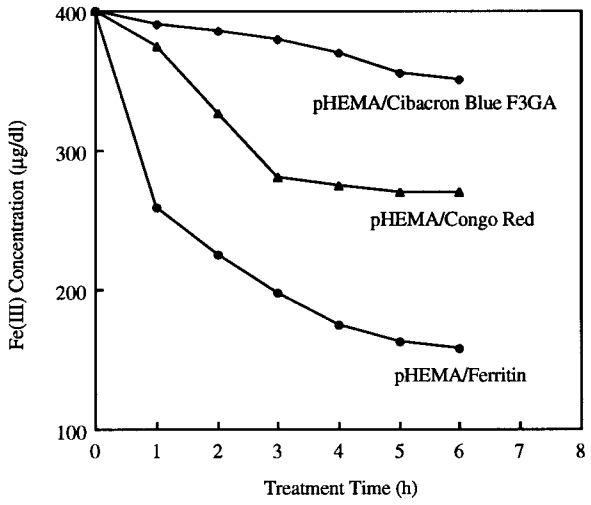

Figure 6 Iron removal rate. The plasma Fe(III) concentration was 400 μg/dL; the cibacron blue F3GA, Congo red, and ferritin loadings were 1.07 μmol/cm^2, 0.80 μmol/cm^2, and 460 μg/cm^2, respectively; and the temperature was 25°C.

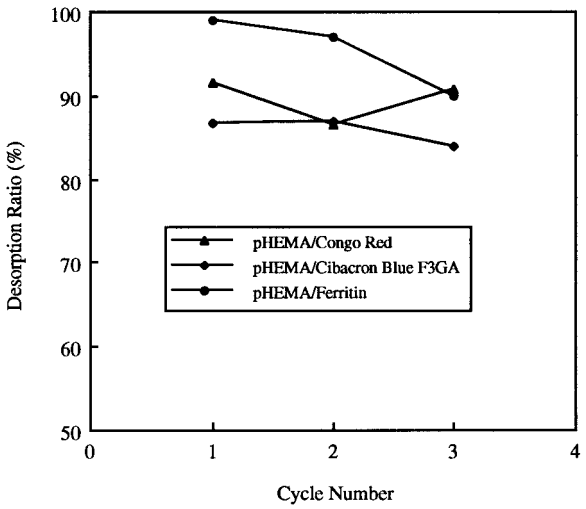

Figure 7 Repeated use of films. The plasma Fe(III) concentration was 400 μg/dL; the cibacron blue F3GA, Congo red, and ferritin loadings were 1.07 μmol/cm^2, 0.80 μmol/cm^2, and 460 μg/cm^2, respectively; and the temperature was 25°C.

concluded that EDTA is a suitable desorption agent that allows the repeated use of the affinity sorbent used in this study.

To verify the reusability of the pHEMA/cibacron blue F3GA, pHEMA/Congo red, and pHEMA/ferritin films, we repeated the adsorption–desorption cycle three times with the same polymeric films. There was no significant loss in the adsorption capacity of all the modified films. The iron adsorption capacity decreased only 10% after three cycles (Fig. 7). It was possible to reuse these affinity sorbents without significant losses in their adsorption capacities.

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