Porous Magnetic Chelator Support for Albumin Adsorption by Immobilized Metal Affinity Separation

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ABSTRACT: Magnetic poly(2-hydroxyethylmethacrylate) (mPHEMA) beads are modified by iminodiacetic acid (IDA) to implify the reactive groups and subsequent binding of Cu^{2+} ions to form metal chelate. mPHEMA beads, in the size range of 80–120 μm, were produced by a modified suspension polymerization technique. mPHEMA beads were characterized by swelling tests, electron spin resonance (ESR), FTIR, and scanning electron microscopy (SEM). Important results obtained in this study are as follows. The swelling ratio of mPHEMA beads was 34%. The presence of magnetite particles in the polymeric structure was confirmed by ESR. FTIR data confirmed that the magnetic polymer beads were modified with functional groups IDA. The mPHEMA beads have a spherical shape and porous structure. The effect of pH and concentration of human serum albumin (HSA), on the adsorption of HSA to the metal-chelated magnetic beads, were examined in a batch reactor. Most importantly, the magnetic beads had little nonspecific adsorption for HSA (0.5 mg/g) before introducing IDA groups. Cu^{2+} chelation increased the HSA adsorption up to 28.4 mg/g. Adsorption behavior can be described at least approximately with the Langmuir equation. Regeneration of the metal-chelated magnetic beads was easily performed with 1.0*M* NaSCN, pH 8.0, followed by washing with distilled water and reloading with Cu^{2+}. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 2501–2510, 2004

Key words: magnetic polymers; separation techniques; chromatography; proteins

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in blood plasma.[1] Research on albumin separation has attracted considerable attention for its great potential in blood protein manufacture. HSA is isolated from human plasma by Cohn's method.[2] Cohn's method concerns precipitation of proteins by using ethanol with varying pHs, ionic strengths, and temperatures. This technique, however, which is the oldest method of industrial fractionation of proteins, is not highly specific and can give partially denatured proteins.[3] Recently, immobilized metal ion affinity chromatography has become a widespread analytical and preparative separation method for therapeutic proteins, peptides, nucleic acids, hormones, and enzymes.[4–14] Many transition metals can form stable complexes with electron-rich compounds (aromatic molecules and olefins) and may coordinate molecules containing O, N, and S by ion dipole interactions. Metal ion ligands are first-row transition metal ions (Zn^{2+}, Ni^{2+}, Cu^{2+}, and Fe^{3+}) incorporated by iminodiacetic acid, nitrilotriacetic acid, amino salicylic acid, and carboxymethylated amino acids.[15–19] The number of histidine residues in the protein is of primary importance in the overall affinity for attached metal ions. In addition, factors such as the accessibility, microenvironment of the binding residue (i.e., the imidazole group of histidine, the thiol group of cysteine, and the indoyl group of tryptophan), cooperation between neighboring amino acid side chains, and local conformations play important roles in biomolecule binding. Aromatic amino acids and the amino-terminus of the peptides also have some contributions.[20] The low cost of metals and the ease of regeneration of adsorbents are attractive features of metal-chelate affinity separation.

The development of the magnetic adsorbents promises to overcome many of the problems associated with chromatographic separations in packed bed and conventional fluidized bed systems.[21] Magnetic adsorbents combine some of the best characteristics of fluidized beds (low pressure drop) and fixed beds (high mass-transfer rates and good fluid–solid contact).[22] Recently, there has been increased interest in the use of magnetic affinity adsorbents in biomolecule coupling and protein purification.[23–26] Magnetic adsorbents can be produced by using both inorganic materials or a number of synthetic and natural polymers. High mechanical resistance, insolubility, and excellent shelf life make inorganic materials ideal as adsorbents. The main disadvantage of inorganic supports is their

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limited functional groups for ligand coupling. Magnetic adsorbents can be porous or nonporous and they are commonly manufactured from polymers because they have a variety of functional groups which can be tailored to use in different applications.[27–32]

This article reports on the preparation and the use of magnetic immobilized metal affinity separation media based on poly(2-hydroxyethyl methacrylate) (mPHEMA), especially when dealing with highly viscous mediums such as blood contact, where the magnetic adsorbent in a magnetically stabilized fluidized bed is desirable because of high convective transport rates without cell damage. Metal-chelating ligand iminodiacetic acid (IDA) was covalently attached to the magnetic beads. The resulting metal–chelate–affinity adsorbent was then tested for its propensity to adsorb HSA in a batch system. The adsorption conditions (i.e., HSA concentration and pH) and the desorption behavior of HSA were investigated.

EXPERIMENTAL

Materials

2-Hydroxyethyl methacrylate (HEMA) was purchased from Sigma (St. Louis, MO, USA) and was purified by vacuum distillation under a nitrogen atmosphere. Ethylene glycol dimethacrylate (EGDMA) was obtained from Fluka AG (Switzerland). Inhibitors were removed by alkaline salt extraction (20% NaCl and 5% NaOH), washed twice with distilled water, dried with $CaCl_2$, and stored at 4°C until use. Magnetite particles (Fe_3O_4, diameter < 5 μm) were obtained from Aldrich (USA). Iminodiacetic acid disodium salt (IDA) was obtained from Aldrich (Munich, Germany) and used without further purification. HSA (98% pure by gel electrophoresis, fatty acid free, 67 kDa) was purchased from Aldrich. Acetic acid and miscellaneous chemicals were of analytical reagent grade and supplied by Merck (Darmstadt, Germany). Coomassie Brilliant blue for the Bradford Protein assay was acquired from Bio-Rad (Richmond, CA, USA). All water used in the experiments was purified by 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.

Preparation of magnetic PHEMA beads

Details of the preparation and characterization of the mPHEMA beads were reported elsewhere.[33,34] A brief description of polymerization procedure was given below: The dispersion medium was prepared by dissolving 200 mg of poly(vinyl alcohol) (PVA; molecular weight: 50,000) within 50 mL distilled water. The desired amount of 2,2′-azobisisobutyronitrile (AIBN; 60 mg) was dissolved in the monomer phase 12.0/4.0/8.0 mL (EGDMA/HEMA/toluene) with 1.0 g magnetite particles. This solution was then transferred into the dispersion medium placed in a glass polymerization reactor (100 mL), which was in a thermostatic water bath. The reactor was flushed by bubbling nitrogen and then sealed. Polymerization medium was stirred magnetically at 600 rpm. The reactor temperature was kept at 65°C for 4 h. Then, the polymerization was completed at 90°C in 2 h. After polymerization, the mPHEMA beads were separated from the polymerization medium. The residuals (e.g., unconverted monomer, initiator, and other ingredients) were removed by an extensive cleaning procedure. Briefly, magnetic beads 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 includes also an activated carbon column, until to be assured that the beads are clean. Purity of the beads were followed by observing the change of optical densities of the samples taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the beads obtained by using a differential scanning microcalorimeter (Mettler, Switzerland). Optical density of the original beads was 2.7. After applying the cleaning procedure, this value was reduced to 0.06. In addition, when the thermogram of the uncleaned bead was recorded, it has a peak ~ 60°C. This peak might be originated from AIBN. After applying this cleaning procedure between 30 and 100°C, any peak was not observed on this thermogram.

Characterization of mPHEMA beads

Water uptake tests

Water uptake properties of the mPHEMA beads were determined by the volumetric method. In this method, the dry beads of a known amount (100 mg) were placed in two cylindrical glass tubes and top points of the tubes were marked. Afterwards, the tubes were filled with distilled water and the beads were allowed to swell at room temperature. The height of the beads was marked periodically (i.e., each 30 min). The height of the swollen beads in the tubes was used to calculate the swelling ratio from the following formula, where h_{swollen} is height of swollen beads and h_{dry} is the height of dry beads

$$\text{Swelling ratio (\%)} = [(h_{\text{swollen}} - h_{\text{dry}})/h_{\text{dry}}] \times 100 \quad (1)$$

Scanning electron microscopy

The surface morphology and bulk structure of the mPHEMA beads were observed in a scanning electron

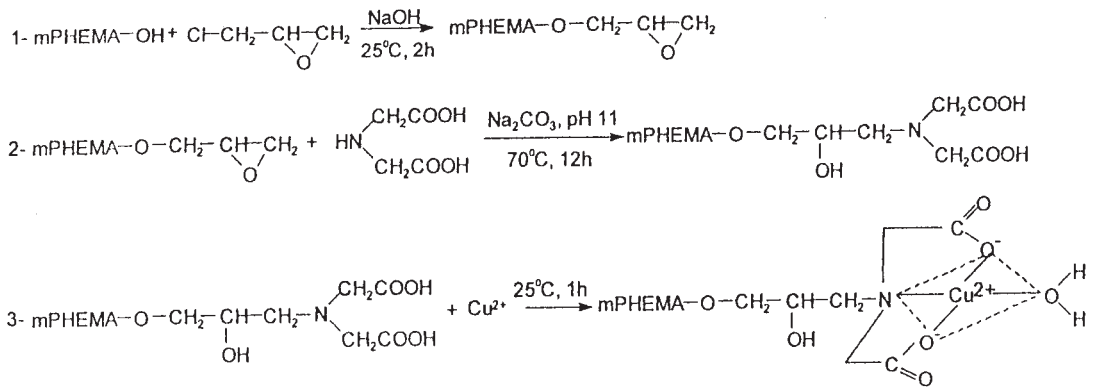


Figure 1 Schematic diagram for the preparation of magnetic PHEMA metal-chelate affinity beads.

microscope (SEM; JEOL, JEM 1200EX, Tokyo, Japan). mPHEMA beads were dried at room temperature and coated with a thin layer of gold (about 100 Å) in vacuum and photographed in the electron microscope with ×1000 magnification. The particle size was determined by measuring at least 100 beads on photographs taken on a SEM.

Analysis of magnetism

The degree of magnetism of the mPHEMA beads was measured in a magnetic field by using a vibrating-sample magnetometer (Princeton Applied Research, model 150A, USA). The presence of magnetite particles in the polymeric structure was investigated with an electron spin resonance (ESR) spectrophotometer (Bruker, USA).

FTIR studies

Infrared spectrum was recorded on a Shimadzu FTIR 8000 Series FTIR spectrophotometer (Japan); 15 scans were taken at 4 cm^{-1} resolution. Polymer beads were prepared by dispersing in dry KBr pellets and recorded between 4000 and 400 cm^{-1}.

Elemental analysis

The amount of IDA attached on the beads was evaluated by using an elemental analysis instrument (Leco, CHNS-932, USA) by considering the nitrogen stoichiometry.

IDA-attached beads

Prior to the activation process, magnetic beads were kept in distilled water for about 24 h and washed on a glass filter with 0.5*M* NaCl solution and water for the system to reach to equilibrium. One gram of mPHEMA beads was incubated in a mixed solution of 2.5 mL epichlorohydrin and 25 mL 2*M* NaOH and 0.1 g $NaBH_4$ at room temperature for 2 h. After incubation, the magnetic beads were rinsed with deionized water. For coupling IDA, the reaction mixture (50 mL of 0.8 g IDA + 2.0*M* $NaCO_3$, pH 11 and epichlorohydrin-conjugated magnetic beads) was incubated at 70°C in a heating mantle under mild stirring for 12 h. After coupling reaction, the magnetic beads were washed with 5% acetic acid and deionized water until the washing solutions were neutral.

Incorporation of Cu^{2+} ions

Chelates of Cu^{2+} ions with IDA-conjugated magnetic beads were prepared as follows: 1.0 g of the IDA-conjugated magnetic beads were mixed with 50 mL of aqueous solution containing 30 ppm Cu^{2+} ions, at a constant pH of 4.1 (adjusted with HCl and NaOH), which was the optimum pH for Cu^{2+} chelate formation and at room temperature. A 1000-ppm atomic absorption standard solution [$Cu(NO_3)_2$ salt containing 10% HNO_3] was used as the source of Cu^{2+} ions. The flasks were stirred magnetically at 100 rpm for 1 h (sufficient to attain equilibrium). The concentration of the Cu^{2+} ions in the resulting solutions was determined with a graphite furnace atomic absorption spectrophotometer (AAS AA800, Perkin–Elmer, Bodenseewerk, Germany). The Cu^{2+} chelation step and other chemical modifications mentioned before (i.e., epichlorohydrin activation and iminodiacetic acid immobilization) were depicted in Figure 1. All instrumental conditions were optimized for maximum sensitivity as described by the manufacturer. For each sample, the mean of 10 AAS measurements was recorded. The amount of adsorbed Cu^{2+} ions was calculated as

$$Q = [(C_O - C_A)V]/m \quad (2)$$

where Q is the amount of Cu^{2+} ions adsorbed (mmol/g); C_0 and C_A are the concentrations of the Cu^{2+} ions in the initial solution and in the equilibrium, respec-

tively (mmol/L); V is the volume of the aqueous phase (L); and m is the amount of beads (g).

Cu^{2+} leakage from the IDA-conjugated magnetic beads was investigated with media pH (4.0–8.0), and also in a medium containing 1.0*M* NaSCN, at pH of 8.0. The magnetic bead suspensions were stirred 24 h at room temperature. Cu^{2+} ion concentration was then determined in the supernatants by using an atomic absorption spectrophotometer. It should also be noted that immobilized metal containing magnetic PHEMA beads were stored at 4°C in the 10 mM Tris–HCl buffer (pH 7.4) with 0.02% sodium azide to prevent microbial contamination.

HSA adsorption from aqueous solutions

In these experiments, the effects of protein concentration and medium pH on the adsorption capacity of IDA-conjugated and Cu^{2+}-chelated magnetic beads were studied. The adsorption experiments were carried out batchwise in the media at different pH values. The pH of the adsorption medium was varied between 4.0 and 8.0 by using different buffer systems (0.1*M* CH_3COONa-CH_3COOH for pH 4.0–6.0, 0.1*M* K_2HPO_4-KH_2PO_4, for pH 7.0, and 0.1*M* Tris–HCl for pH 8.0). HSA concentration was varied between 0.5 and 5.0 mg/mL. In a typical adsorption experiment, HSA was dissolved in 10 mL of buffer solution, and 100 mg of beads was added. Then, the adsorption experiments were conducted for 2 h (equilibrium time) at 4°C at a stirring rate of 100 rpm. At the end of this equilibrium period, HSA adsorption by the modified beads was determined by measuring the initial and final concentration of HSA within the adsorption medium by using Coomassie Brilliant blue as described by Bradford.[35]

Regeneration studies

HSA desorption experiments were performed in a buffer solution containing 1.0*M* NaSCN at pH 8.0. HSA adsorbed magnetic beads were placed in the desorption medium and stirred for 1 h at 25°C, at a stirring rate of 100 rpm. The final HSA concentration within the desorption medium was determined by spectrophotometry. In the case of Cu^{2+} carrying adsorbents, desorption of Cu^{2+} ions were also measured in the desorption media by means of an atomic absorption spectrophotometry. The desorption ratio was calculated from the amount of HSA adsorbed on the magnetic beads and the amount of HSA desorbed by using the expression:

$$\text{Desorption ratio (\%)} = \frac{\text{amount of HSA desorbed}}{\text{amount of HSA adsorbed on the beads}} \times 100 \quad (3)$$

To check the reusability of the Cu^{2+}-chelated magnetic PHEMA/IDA beads, HSA adsorption–desorption operation was done five times by using the same adsorbent. After each HSA adsorption–desorption experiment, the Cu^{2+} ions was stripped with 25 mM EDTA at pH 4.9, and the Cu^{2+} loading procedure was applied again.

HSA adsorption from human plasma

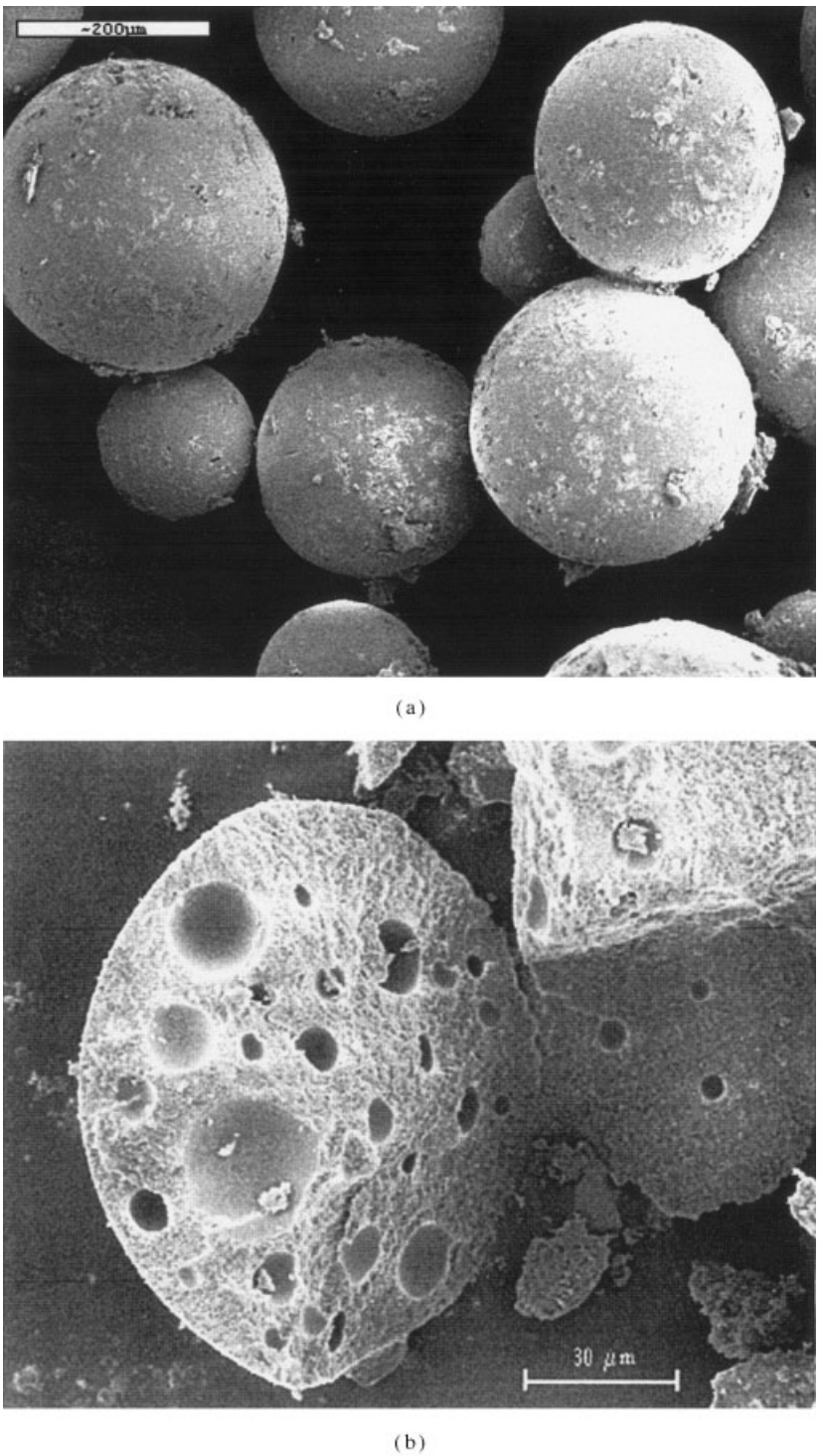
HSA adsorption from human plasma with the Cu^{2+}-chelated magnetic PHEMA/IDA beads was studied batchwise. The blood was obtained from a healthy human donor. Blood samples were centrifuged at 500 *g* for 3 min at room temperature to separate the plasma. The original plasma of the donor contained 42.0 mg HSA/mL as determined by bromocresol green (BCG) dye method at 628 nm.[36] Ten milliliters of the freshly separated human plasma was incubated with 100 mg beads preequilibrated with acetate buffer (pH 5.0) for 2 h. These experiments were conducted at room temperature and a stirring rate of 100 rpm. The amount of HSA adsorbed was determined by measuring the initial and final concentration of HSA in plasma. Phosphate-buffered saline (PBS; pH 7.4, NaCl: 0.9%) was used for dilution of human plasma.

To show specificity of the Cu^{2+}-chelated magnetic PHEMA/IDA beads, adsorptions of other proteins, namely fibrinogen and γ-globulin, were also studied. The metal-chelated beads were incubated with a human plasma containing albumin (42.0 mg/mL), fibrinogen (2.0 mg/mL), and γ-globulin (16.1 mg/mL) at room temperature for 2 h. Total protein concentration was measured by using the total protein reagent (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK; Catalog ref. no. 712076) at 540 nm, which was based on Biuret reaction.[36] Chronometric determination of fibrinogen according to the Clauss method on plasma was performed by using Fibrinogene-Kit (ref. no. 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France).[37] HSA concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd.; Catalog ref. no. 229241), which was based on BCG dye method.[36] γ-Globulin concentration was determined from the difference.

RESULTS AND DISCUSSION

Characteristics of mPHEMA beads

The metal-chelate adsorbents were extensively characterized to obtain water content, SEM, FTIR spectrum, magnetism, and elemental analysis. The equilibrium water content of mPHEMA beads was 34%, indicating the magnetic beads present a hydrophilic skeleton. The SEM photographs of the magnetic beads are



(a)

(b)

Figure 2 SEM micrographs of mPHEMA beads. (A) surface; (B) cross section.

shown in Figure 2. The shape and surface structure of the magnetic beads can be clearly seen and show that the beads have a spherical form and rough surface due to the abrasion of magnetite crystals (diameter < 0.1 μm) during the polymerization. The photograph in Figure 2(B) was taken with broken beads to observe the internal part. The presence of macropores within the bead interior is clearly seen in this photograph. It can be concluded that the mPHEMA beads have a macroporous interior surrounded by a reasonably rough surface in the dry state. The roughness of the bead surface should be considered as a factor providing an increase in the surface area. In addition, these macropores reduce diffusional resistance and facilitate

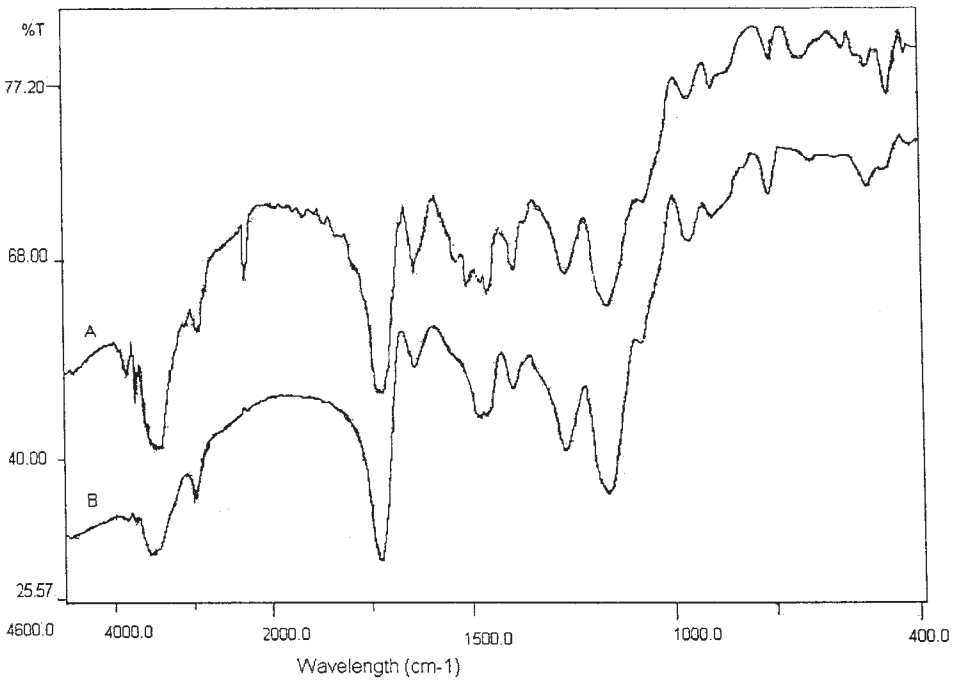


Figure 3 FTIR spectrums: (A) magnetic PHEMA; (B) IDA conjugated magnetic PHEMA beads.

mass transfer because of the high surface area. This also provides higher ligand coupling and enhances protein adsorption capacity.

The presence of magnetite particles in the polymeric structure was confirmed by ESR. mPHEMA beads have a relative intensity of 400. This value shows that polymeric structure has a local magnetic field because of magnetite in its structure. The magnetic property of the polymeric structure was also expressed in electron mass unit (EMU), showing the behavior of magnetic beads in a magnetic field by using a vibrating magnetometer. A magnetic field of 2250 G was found sufficient to excite all of the dipole moments present in 1.0 g mPHEMA sample. These data will be an important design parameter for a magnetically stabilized fluidized bed or for magnetic affinity filtration system by using these magnetic beads. The value of the magnetic field required to stabilize the fluidized bed is a function of the flow velocity, particle shape, size, size distribution, and magnetic susceptibility of beads. In the literature, this value was found to change from 8 to 20 kG for various applications.[38] Thus, mPHEMA beads will need less magnetic intensity in a magnetically stabilized fluidized bed or for a magnetic filter affinity system.

FTIR spectrums were undertaken to determine the structure of the magnetic PHEMA and the IDA-conjugated magnetic PHEMA beads (Fig. 3). The FTIR spectrum of IDA-conjugated magnetic PHEMA beads with characteristic peaks appear at 3560 cm^{-1} (characteristic hydroxyl, OH stretching vibration), 2924 cm^{-1} (CH_3 stretching vibration), 2363 cm^{-1} (C—N stretching vibration), and 1738 cm^{-1} (carbonyl stretching vibration) [Fig. 3(B)]. The N—H peak appearing at 3647 cm^{-1} is associated with the IDA. These data confirmed that the magnetic polymer beads were modified with functional groups IDA.

Metal-chelating ligand IDA is covalently attached on epichlorohydrin-activated magnetic PHEMA beads via the reaction between the epoxide groups of the epichlorohydrin and the primer amine groups of the IDA. The highest IDA surface density obtained was 327 μmol IDA/g polymer. The studies of IDA leakage from the mPHEMA beads showed that there was no IDA leakage in any medium used throughout this study, even in a long storage period (more than 40 weeks).

The amount of Cu^{2+} present in the magnetic beads was 320 μmol/g of magnetic beads (as determined by atomic absorption spectroscopy). Note that the binding ratio of Cu^{2+} ions to conjugated IDA molecules was approximately 1.

Albumin adsorption studies

Effects of pH

Figure 4 shows the effects of pH. In all the cases investigated, the maximum adsorption of HSA was observed at pH 7.0. Significantly lower adsorption capacities were obtained with magnetic beads in more acidic and in more alkaline pH regions. The decrease in the HSA adsorption capacity in more acidic and more alkaline pH regions can be attributed to electro-

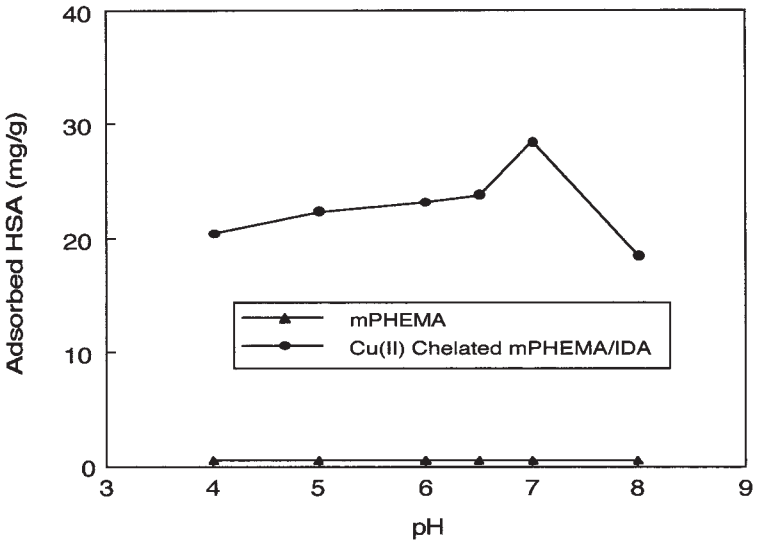


Figure 4 Effects of pH on adsorption of HSA. IDA incorporation: 327 μmol/g; Cu^{2+} loading: 320 μmol/g; initial concentration of HSA: 2.0 mg/mL; T: 25°C.

static repulsion effects between the opposite charged groups. It has been shown that proteins have no net charge at their isoelectric points, and therefore, the maximum adsorption from aqueous solutions is usually observed at their isoelectric points. The isoelectric pH of HSA is 5.0. In this study, the maximum adsorption pH shifted to 7.0. Notably, the affinity between HSA molecules (especially imidazole group of the histidine residue, thiol group of cysteine, and indoyl group of tryptophan) and attached Cu^{2+} ions is primarily the specific or electron donor–acceptor interactions. These specific interactions may be resulted from the deprotonation of amino acid side changes (e.g., histidine and especially primary amines such as lysine) of HSA with immobilized Cu^{2+} ions. The conformational changes of albumin molecules due to specific interactions at this pH may also contribute the specific interaction.

Effects of albumin concentration

Adsorption isotherms were measured at near neutrality at which pH of the surface histidines are unprotonated and free to coordinate bound metal ions.[39] The relationship between the HSA adsorption and the equilibrium concentration of HSA is given in Figure 5. With the increase of the concentration of HSA, the adsorption capacity increased, below about 2.0 mg/

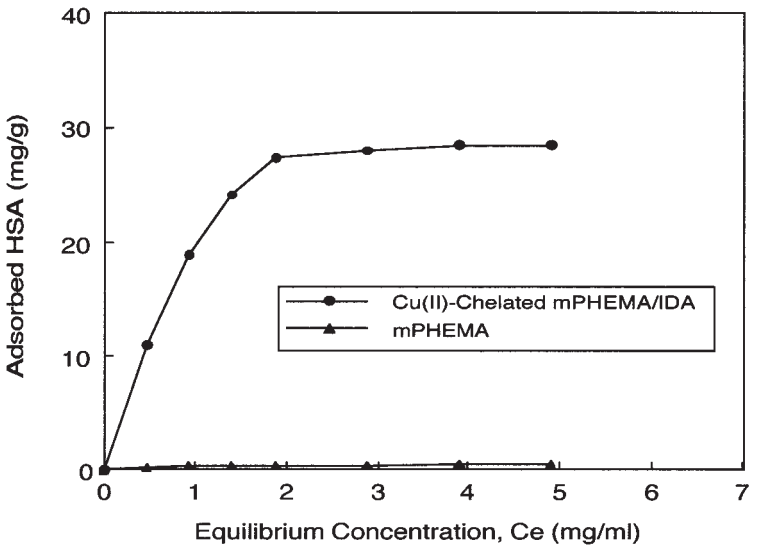


Figure 5 Effects of albumin initial concentration on adsorption of HSA. IDA incorporation: 327 μmol/g; Cu^{2+} loading: 320 μmol/g; pH: 7.0; T: 25°C.

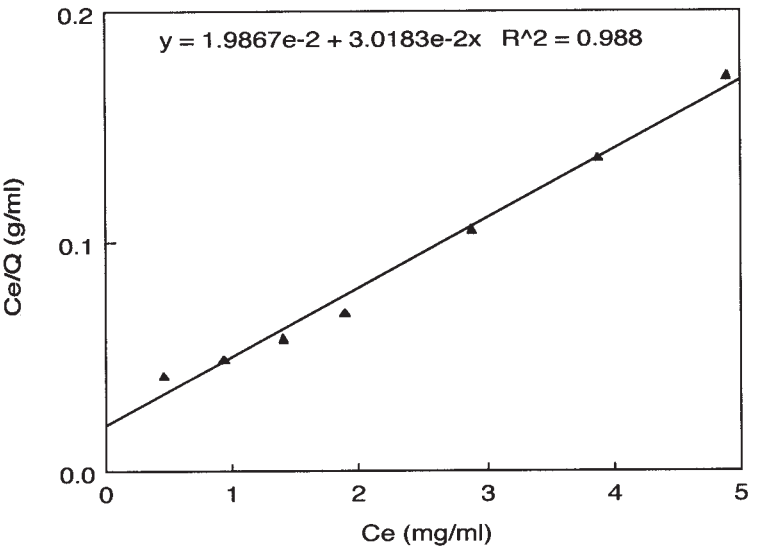


Figure 6 Linear representation of Langmuir equation of HSA with Cu^{2+}-chelated mPHEMA/IDA beads. IDA incorporation: 327 μmol/g; Cu^{2+} loading: 320 μmol/g; pH: 7.0; T: 25°C.

mL, and then reached a saturation level for the Cu^{2+} chelated magnetic beads. It is of importance for a good affinity matrix to have little nonspecific adsorption for proteins. The nonspecific HSA adsorption was very low (0.5 mg/g) due to the highly hydrophilic property of the magnetic bead surface. It is evident that Cu^{2+} loading increased the HSA adsorption capacity of the beads (28.4 mg/g). This is due to the fact that a strong specific interaction especially between imidazole side chains of histidine residue of HSA and chelated Cu^{2+} ions. It should be reported that different interaction mechanisms of metal affinity separations with proteins have been proposed,[40–42] but still the macromolecular recognition of protein with immobilized metal ions obviously remains unclear. In one proposed mechanism, the formation of a coordination complex structure between protein and immobilized metal ion is considered to be the major binding manner. However, more than one type of interaction mechanism are operational. Factors influencing the interactions include the number of electron-donating groups on the protein surface, pH, concentration, type of metal ion, ligand density, and structure of chelating ligand.

The Langmuir adsorption model was found to be applicable in interpreting HSA adsorption by Cu^{2+}-chelated mPHEMA/IDA beads. The Langmuir adsorption isotherm is expressed by eq. (4). The corresponding transformations of the equilibrium adsorption data for HSA gave rise to a linear plot, indicating that the Langmuir model could be applied in this system and described by

$$Q = Q_{max}C_{eq}/(K_d + C_{eq}) \quad (4)$$

where Q is the adsorbed amount of HSA (mg/g), C_{eq} is the equilibrium HSA concentration in solution (mg/mL), K_d is the dissociation equilibrium constant (ml/mg), and Q_{max} is the maximum HSA adsorption capacity (mg/g). This equation can be linearized so that

$$1/Q = [1/Q_{max}K_d)][1/C_{eq}] + [1/Q_{max}] \quad (5)$$

The plot of $1/C_{eq}$ versus $1/Q$ was employed to generate the intercept of $1/Q_{max}$ and the slope of $1/Q_{max}K_d$ (Fig. 6). The maximum HSA adsorption capacity (Q_{max}) was obtained from experimental data. The correlation coefficient (R^2) was found to be very high (0.988) for Cu^{2+}-chelated mPHEMA/IDA beads, indicating that the Langmuir adsorption model can be applied in this metal-chelating affinity adsorbent system. It should be also noted that the maximum adsorption capacity (Q_{max}) and the dissociation equilibrium constant (K_d) were found to be 50 mg/g and 0.67 mL/mg from the Langmuir adsorption model.

Regeneration of the beads

Regeneration is a crucial step in all affinity chromatography techniques. It was thus necessary to evaluate the regeneration efficiency of the affinity adsorbents after each cycle. In this study, >90% of the adsorbed HSA molecules were removed easily from the magnetic beads in all cases when NaSCN was used for desorption. Note that there was no Cu^{2+} release in this case, which shows that Cu^{2+} ions are attached to IDA groups on the beads' surface by strong chelate formation. With the desorption data given above, we concluded that NaSCN is a suitable desorption agent for the Cu^{2+}-chelated IDA modified magnetic beads. To examine the effects of desorption conditions on albumin denaturation, fluorescence spectrophotometry

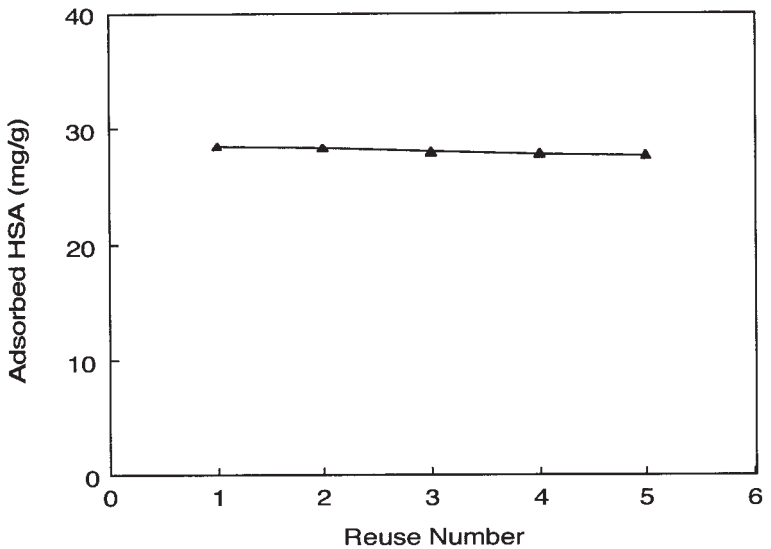


Figure 7 Repeated use of magnetic beads. IDA incorporation: 327 μmol/g; Cu^{2+} loading: 320 μmol/g; initial concentration of HSA: 2.0 mg/mL; pH: 7.0; T: 25°C.

was employed. The fluorescence spectra of albumin samples obtained from the desorption step were recorded. The fluorescence spectrum of native albumin was taken. On the other hand, fluorescence spectra of the samples withdrawn from the desorption step were very close to those of native albumin and no significant shift of maximum wavelength was detected in the spectra of these samples relative to that of native albumin. It may be concluded that metal-chelate affinity chromatography with magnetic PHEMA/IDA beads can be applied for albumin separation without causing any denaturation.

To show the reusability of the Cu^{2+}-chelated magnetic PHEMA/IDA beads, the adsorption–desorption cycle was repeated five times by using the same modified magnetic beads from aqueous protein solution. As seen in Figure 7, there was no significant loss in the adsorption capacity of the magnetic beads. Moreover, no obvious changes of the magnetic properties of the PHEMA beads were found in the recycling process. It should be also noted that these operations did not result in any damage to the morphology of the magnetic beads, when examined visually. These results demonstrated the stability of the present solid support as a magnetic adsorbent.

HSA adsorption from human plasma

Table I shows the adsorption for human serum obtained from a healthy donor. There was a very low nonspecific adsorption of HSA (1.4 mg/g) on the untreated magnetic PHEMA beads, while much higher adsorption values (48.6 mg/g) were obtained when the Cu^{2+}-chelated magnetic PHEMA/IDA beads were used. It is worth noting that adsorption of HSA onto the Cu^{2+}-chelated magnetic PHEMA/IDA beads was $\sim$ 1.7-fold higher than those obtained in the studies in which aqueous solutions were used. This may be explained as follows: the conformational structure of the HSA molecule within its native environment (i.e., human plasma) is much more suitable for specific interaction with the Cu^{2+}-chelated magnetic PHEMA/IDA beads. The high HSA concentration (42.0 mg/mL) may also contribute to this high adsorption due to the high driving force between the aqueous (i.e., human plasma) and solid phases (i.e., magnetic beads).

To show the specificity of the magnetic beads, competitive protein adsorption was also studied. Interesting results were obtained in these studies. Adsorption capacities were obtained as 48.6 mg/g for albumin, 2.7 mg/g for fibrinogen, and 6.3 mg/g for γ-globulin. The total protein adsorption was determined as 58.2 mg/g. It is worth noting that adsorption of other plasma proteins (i.e., fibrinogen and γ-globulin) on the Cu^{2+}-chelated magnetic PHEMA/IDA beads are negligible. It should be noted that albumin is the most abundant protein in plasma. It generally makes up more than half of the total plasma proteins. It may be resulted that this low adsorption of fibrinogen and γ-globulin is due to the high concentration of albumin.

TABLE I
HSA Adsorption from the Plasma of a Healthy Donor

HSA concentration (mg/mL)	Amount of HSA adsorbed (mg/g)
5.3	33.1
10.5	37.4
21.0	41.9
42.0	48.6

T: 25°C; plasma volume: 40 mL.

CONCLUSION

Conventional chromatographic techniques are time consuming because of mass transfer limitations and high pressure drops. Magnetic adsorbent technology enables the use of magnetic processing for rapid and selective separation. Magnetic separation has several potential advantages over conventional approaches. Magnetically stabilized fluidized bed cartridges require high flow rates with a much lower operating pressure than a packed bed column. In this technique, the biomolecule to be separated can be directly transported by convection to the ligand coupled on the surface of the beads, higher throughput, and faster processing times can be achieved. Magnetic PHEMA beads were produced by suspension polymerization of HEMA. mPHEMA beads with covalently attached IDA was developed for metal-chelate affinity adsorption of HSA. The results obtained in this article show that adsorption of HSA under different pH values and HSA concentration have revealed the affinity of Cu^{2+}-chelated mPHEMA beads for HSA. It was possible to use the magnetic beads in a HSA adsorption–desorption cycle.

References

1. Norbert, W. Fundamental of Clinical Chemistry; W. B. Saunders: London, 1976.
2. Cohn, E. J.; Strong, L. E.; Hughes, W. L.; Mulford, D. J.; Ashworth, J. N.; Melin, M.; Taylor, H. L. J Am Chem Soc 1946, 68, 459.
3. Stotz, J. F.; Rivat, C.; Geschier, C.; Colosett, P.; Streiff, F. Swiss Biotech 1990, 8.
4. Gupta, M. N.; Jain, S.; Roy, I. Biotechnol Prog 2002, 18, 18.
5. Ivanov, A. E.; Galaev, I. Y.; Kazakov, S. V.; Mattiasson, B. J Chromatogr A 2001, 907, 115.
6. Abudiab, T.; Beitle, R. R. J Chromatogr A 1998, 795, 211.
7. Kim, Y. J. Biotechnol Techn 1999, 13, 837.
8. Denizli, A.; Pişkin, E. J Chromatogr A 1996, 731, 57.
9. Denizli, A.; Koktürk, G.; Kozluca, A.; Pişkin, E. J Appl Polym Sci 1997, 63, 27.
10. Denizli, A.; Pişkin, E. J Appl Polym Sci 1997, 65, 2085.
11. Gaberc-Porekar, V.; Menart, V. J Biochem Biophys Methods 2001, 49, 335.
12. Arıca, Y.; Testereci, H.. N.; Denizli, A. J Chromatogr A 1998, 799, 83.
13. Luo, Q.; Zou, H.; Xiao, X.; Guo, Z.; Kong, L.; Mao, X. J Chromatogr A 2001, 926, 255.
14. Denizli, A.; Şenel, S.; Arıca, M. Y. Colloids Surf B 1998, 11, 113.
15. Denizli, A.; Denizli, F.; Pişkin, E. J Biomater Sci Polym Ed 1999, 10, 305.
16. Denizli, F.; Denizli, A.; Arıca, Y. Polym Int 19699, 48, 360.
17. Denizli, A.; Yavuz, H.; Garipcan, B.; Arıca, Y. J Appl Polym Sci 2000, 76, 115.
18. Denizli, A.; Yavuz, H.; Arıca, Y. Colloids Surf A 2000, 174, 307.
19. Patwardhan, A.; Goud, G.; Koepsel, R.; Ataai, M. J Chromatogr A 1997, 787, 91.
20. Yip, T. T.; Nakagawa, Y. Porath, J. Anal Biochem 1989, 183, 159.
21. Hultman, T.; Stahl, S.; Hornes, E.; Uhlen, M. Nucleic Acids Res 1989, 17, 4937.
22. Halling, P. J.; Dunhill, M. D. Enzyme Microbial Technol 1980, 2, 136.
23. Tong, X. T.; Xue, B.; Sun, Y. Biotechol Prog 2001, 17, 134.
24. Xue, B.; Tong, X. D.; Sun, Y. Sep Sci Technol 2001, 36, 2449.
25. Xue, B.; Sun, Y. J Chromatogr A 2001, 921, 109.
26. Tong, X. D.; Sun, Y. Biotechol Prog 2001, 17, 738.
27. Denizli, A. J Chromatogr A 1998, 793, 47.
28. Takahashi, K.; Kodera, Y.; Milhama, T. Biochem Biophys Res Commun 1987, 142, 291.
29. Balto, B. A.; J Macromol Sci Chem A 1980, 14, 107.
30. Cocker, T. M.; Fee, C. J.; Evans, R. A. Biotechnol Bioeng 1997, 53, 79.
31. Chetty, A. S.; Burns, M. A. Biotechnol Bioeng 1991, 38, 963.
32. Arıca, Y.; Yavuz, H.; Patır, S.; Denizli, A. J Mol Catal B 2000, 11, 127.
33. Odabaşı, M.; Denizli, A. J Chromatogr B 2001, 760, 137.
34. Denizli, A.; Say, R. J Biomater Sci Polym Ed 2001, 12, 1059.
35. Bradford, M. M. Anal Biochem 1976, 72, 248.
36. Tietz, N. W. Textbook of Clinical Chemistry; W.B. Saunders: Philadelphia, 1986; p. 589.
37. Clauss, A. Acta Haematol 1957, 17, 237.
38. Wang, Y. M.; Wang, Y. X.; Feng, L. X. J Appl Polym Sci 1997, 64, 1843.
39. Todd, R. J.; Haymore, B. L.; Arnold, F. H. Proteins Struct Funct Genet 1991, 10, 156.
40. Sulkowski, E. Trends Biotechnol 1985, 3, 1.
41. Hutchens, T. W.; Yip, T. T. J Inorg Biochem 1991, 42, 105.
42. Hemdan, E. S.; Porath, J. J Chromatogr 1985, 323, 255.