Metal-Chelated Polyamide Hollow Fibers for Human Serum Albumin Separation

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ABSTRACT: We modified microporous polyamide hollow fibers by acid hydrolysis to amplify the reactive groups and subsequent binding of Cibacron Blue F3GA. Then, we loaded the Cibacron Blue F3GA-attached hollow fibers with different metal ions (Cu^{2+}, Ni^{2+}, and Co^{2+}) to form the metal chelates. We characterized the hollow fibers by scanning electron microscopy. The effect of pH and initial concentration of human serum albumin (HSA) on the adsorption of HSA to the metal-chelated hollow fibers were examined in a batch system. Dye- and metal-chelated hollow fibers had a higher HSA adsorption capacity and showed less nonspecific protein adsorption. The nonspecific adsorption of HSA onto the polyamide hollow fibers was 6.0 mg/g. Cibacron Blue F3GA immobilization onto the hollow fibers increased HSA adsorption up to 147 mg/g. Metal-chelated hollow fibers showed further increases in the adsorption capacity. The maximum adsorption capacities of Co^{2+}-, Cu^{2+}-, and Ni^{2+}-chelated hollow fibers were 195, 226, and 289 mg/g, respectively. The recognition range of metal ions for HSA from human serum followed the order: Ni(II) > Cu(II) > Co(II). A higher HSA adsorption was observed from human serum (324 mg/g). A significant amount of the adsorbed HSA (up to 99%) was eluted for 1 h in the elution medium containing 1.0*M* sodium thiocyanide (NaSCN) at pH 8.0 and 25 m*M* ethylenediaminetetraacetic acid at pH 4.9. Repeated adsorption–desorption processes showed that these metal-chelated polyamide hollow fibers were suitable for HSA adsorption. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 3346–3354, 2002

Key word: polyamides; fibers; protein separation; metal-chelated adsorbents; albumin

INTRODUCTION

Albumin, immunoglobulins, and clotting factors are the most important proteins to the process of obtaining human plasma. Human serum albumin (HSA) represents approximately 50% of all sales of therapeutic plasma protein products.[1] HSA is the most abundant protein in serum. It has many important physiological functions that contribute significantly to colloid osmotic blood pressure and that aid in the transport, distribution, and metabolism of many endogeneous and exogeneous substances, including bile acids, bilirubin, fatty acids, amino acids, steroids, metal ions, and numerous pharmaceuticals.[2,3] Research on protein purification has attracted considerable attention for its great potential in blood protein manufacture.[4] Cohn's method concerns precipitation of proteins with ethanol with varying pH, ionic strength, and temperature. Although the Cohn procedure for the fractionation of albumin and other serum proteins is still widely used by industry, with the advent of improved methods of protein purification there has been a continuous search during the past decades for more efficient protocols, particularly those that employ novel chromatographic techniques[5] because Cohn's method is not specific and can give partially denatured proteins.[6]

Dye and metal chelate affinity adsorbents have been increasingly used for protein purification.[7–10] These adsorbents offer several advantages over group-specific adsorbents in terms of economy, ease of immobilization, stability, and high adsorption capacity.[11] Metal chelate affinity chromatography is of high selectivity because it exploits the affinity of proteins and enzymes for the coordination sites of first-series transition-metal ions. In metal chelate affinity chromatography, the exposed electron-donating amino acid residues on the protein surface, such as the imidazole group of histidine, the thiol group of cysteine, and the indoyl group of tryptophan, contribute to the binding of proteins to immobilized metal ions. This technique also provides the durability (physical and chemical stability), low operating expense, and versatility of conventional chromatographic techniques.[12–14] Different metal ions can be chelated on the affinity adsorbent and can be easily removed for regeneration by a strong chelator such as ethylenediaminetetraacetic acid (EDTA) hundreds of times over period of years after they are stripped off the column without any detectable loss of metal chelate properties.

In recent years, the development of alternative porous supports have received considerable interest. Mi-

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croporous membranes in the form of flat-sheet systems and stacks of membranes, hollow fibers, and radial flow cartridges have been successfully modified; various ligands have been coupled and used for different appications.[15–20] These supports have the advantages of a large surface area, short diffusion path, and low pressure drop. An ideal membrane for protein separation must fulfill the requirements of high hydrophilicity and low nonspecific adsorption, large pore size, chemical and mechanical stability, and a sufficient quantity of reactive groups.[21] Polyamide hollow fibers offer narrow pore size distribution, but because of low concentration of primary amine functional groups available in their structure, they have too low ligand density. These problems could be solved by hydration and binding with a polyhydroxyl-containing materials.[22]

In this study, dye and metal chelate affinity hollow fibers with polyamide as a support matrix were prepared. The dye ligand Cibacron Blue F3GA was covalently attached to polyamide hollow fibers and was used as a metal chelate ligand. The divalent metal ions Ni^{2+}, Co^{2+}, and Cu^{2+} were chelated on the dye–ligand, and the resulting affinity adsorbent was then tested for its propensity to adsorb HSA in a batch system. The adsorption conditions (i.e., initial HSA concentration and pH) and the desorption behavior of HSA were investigated.

EXPERIMENTAL

Materials

Commercially available polyamide hollow fiber was used as a basic matrix for protein adsorption (Akzo; Wuppertal, Germany). HSA (98% pure by gel electrophoresis, fatty acid free, 67 kDa) was supplied from Sigma Chemical Co. (St. Louis, MO) and was used as received. Cibacron Blue F3GA was obtained Polyscience (Warrington, PA) and was used without further purification. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). 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 purified water had a specific conductivity of 18 mΩ/cm.

Methods

Cibacron blue F3GA-attached hollow fibers

Polyamide hollow fibers were exposed for partial hydrolysis under conditions that did not destroy mechanical integrity. Then, Cibacron Blue F3GA was covalently attached to the hollow fibers via a nucleophilic substitution reaction between the chloride of its triazine ring and the amine groups of the polyamide hollow fibers as follows: The hollow fibers were cut to small pieces (1 cm in length) and stirred magnetically at 100 rpm with 3*M* HCl at a constant temperature of 30°C for 20 min. We then arrested the acid hydrolysis by washing with cold water (4°C). The hydrated fibers were then stirred magnetically at 400 rpm in a sealed reactor containing 100 mL of aqueous solution containing 300 mg of Cibacron F3GA for 30 min at 60°C. This was followed by the addition of 7.0 g of NaCl to stimulate the deposition of the dye on the surface of the hollow fiber. After 30 min, 1.0 g of sodium carbonate (Na_2CO_3) was added to accelerate the reaction between the dye and hollow fiber at 80°C for 4 h. After incubation, the Cibacron Blue F3GA-attached hollow fibers were exposed to an extensive washing procedure to avoid any further release. The hollow fibers were first washed with deionized water and then dispersed in methanol and sonicated for 2 h in an ultrasonic bath (200 watt, Bransonic 200, Danbury, CT) to remove the nonspecifically attached/or diffused dye molecules into the pores. At the last stage, hollow fibers were washed again with deionized water and then stored at 4°C with 0.02% sodium azide until use.

The dye leakage from the dye-attached hollow fibers was investigated at different pH values in the range of 4.0–8.0 in the same media that was used in the HSA adsorption experiments given later. Also, Cibacron Blue F3GA leakage was determined in the medium containing 0.5*M* sodium thiocyanide (NaSCN) and 25 m*M* EDTA at pH of 8.0, which was the medium used for the HSA experiments. The medium with the Cibacron Blue F3GA-attached hollow fibers was incubated at room temperature for 24 h. Then, hollow fibers were removed from the medium, and the dye concentration in the supernatant was measured by spectrophotometry at 630 nm.

Characterization of hollow-fiber membranes

Elemental analysis. We evaluated the amount of covalently immobilized Cibacron Blue F3GA on the hollow fibers with an elemental analysis instrument (LECO, CHNS-932, Chicago, IL) by considering the sulfur stoichiometry.

Scanning electron microscopy (SEM) studies. The surface and bulk structures of the hollow fibers were examined with a scanning electron microscope (Raster Electron Microscopy, Leitz-AMR-1000, Köln, Germany).

Porosity measurements. Pore volumes and average pore diameters greater than 20 Å were determined with a mercury porosimeter up to 2000 kg/cm^2 with a Carlo Erba model 200 (Milan, Italy). The surface area of the hollow-fiber sample was measured with a sur-

face area apparatus (the Brunner Emmet Teller (BET) method).

Chelation of metals ions

Adsorption of Cu^{2+}, Co^{2+}, and Ni^{2+} ions from the single aqueous solutions was investigated in batch adsorption-equilibrium experiments. Metal ion solutions (50 mL) were treated with the Cibacron blue F3GA-attached hollow fibers (total length = 50 cm, total mass = 50 mg). The flasks were magnetically stirred at 600 rpm at room temperature. Initial concentration of metal ions were 30 ppm for Cu^{2+} ions and 50 ppm for both Co^{2+} and Ni^{2+} ions. Media pH's were 4.0 for Cu^{2+} ions and 5.5 for both Co^{2+} and Ni^{2+} ions. Copper nitrate [$Cu(NO_3)_2$], nickel nitrate [$Ni(NO_3)_2$], and cobalt nitrate [$Co(NO_3)_2$] were used for preparation of the standard metal ion solutions. After a predetermined equilibrium time (2 h), the hollow fibers were separated from the adsorption medium, and the residual concentration of the metal ions in the aqueous phase was measured with a graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Jena, Germany). Deuterium background correction was used. Pyrolitic-graphite-coated tubes were used for atomic absorption spectrophotometry measurements. All instrumental conditions were optimized for maximum sensitivity as described by the manufacturer. Each sample was read three times, and a mean value and relative standard deviation was computed. Calibrations were performed in the range of analysis, and a correlation coefficient for the calibration curve of.98 or greater was obtained. The instrument response was periodically checked with known metal standard solutions. Adsorption values (μmol/g) were calculated as the difference between initial and final metal ion concentrations. To eliminate contamination, we washed all glassware and plasticware with 0.1*M* HNO_3 and rinsed them with deionized water.

HSA adsorption from aqueous solutions

The HSA 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 with different buffer systems (0.1*M* CH_3COONa–CH_3COOH for pH 4.0–5.0, 0.1*M* K_2HPO_4–KH_2PO_4 for pH 6.0–7.0, and 0.1*M* $NaHCO_3$–H_2CO_3 for pH 8.0). HSA concentration was varied between 1.0 and 7.0 mg/mL. In a typical adsorption experiment, HSA was dissolved in 50 mL of buffer solution, and 100 mg of polymer matrix was added. Then the adsorption experiments were conducted for 2 h (equilibrium time) at 25°C at a stirring rate of 100 rpm. At the end of this equilibrium period, HSA adsorption by metal-chelated hollow fibers was determined by measurement of the initial and final concentration of HSA within the adsorption medium with Coomassie Brilliant Blue as described by Bradford.[23]

HSA adsorption from human serum

HSA adsorption from human serum with dye-attached and metal-chelated hollow fibers 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 serum. The original serum of the donor contained 39.2 mg of HSA/mL as determined by the bromocresol green dye method at 628 nm.[24,25] The freshly separated human serum (20 mL) was incubated with 100 mg of hollow fibers pre-equilibrated with phosphate buffer (pH = 7.4) for 2 h. These experiments were conducted at 25°C and at a stirring rate of 100 rpm. The amount of HSA adsorbed by hollow fibers was determined by measurement of the initial and final concentrations of HSA in serum. Phosphate-buffered saline (pH = 7.4, containing 0.9% NaCl) was used for the dilution of human serum.

Desorption studies and repeated use

The HSA desorption experiments were performed in a buffer solution containing 0.5*M* NaSCN at pH 8.0 or 25 m*M* EDTA at pH 4.9. The HSA adsorbed hollow fibers were placed in the desorption medium with continuous stirring at 100 rpm at 25°C for 1.0 h. The final HSA concentration in desorption medium was determined by spectrophotometry. In the case of different metal-chelated hollow fibers, the release of Co^{2+}, Cu^{2+}, and Ni^{2+} ions were also measured in the desorption media by means of the atomic absorption spectrophotometer. The desorption ratios of HSA and each metal ion were calculated with the following expression:

$$\text{Desorption ratio} = (\text{Amount of HSA desorbed}/\text{Amount of HSA adsorbed}) \times 100 \quad (1)$$

To determine the reusability of the hollow-fiber adsorption and desorption, the cycle was repeated 10 times with the same-affinity hollow fibers.

RESULTS AND DISCUSSION

Properties of polyamides hollow fibers

An ideal matrix for protein separation must have the following requirements: high hydrophilicity and low nonspecific adsorption, a fairly large pore size and a narrow pore size distribution, chemical and mechanical stability, and enough functional reactive groups. Commercially available polyamide hollow fibers may

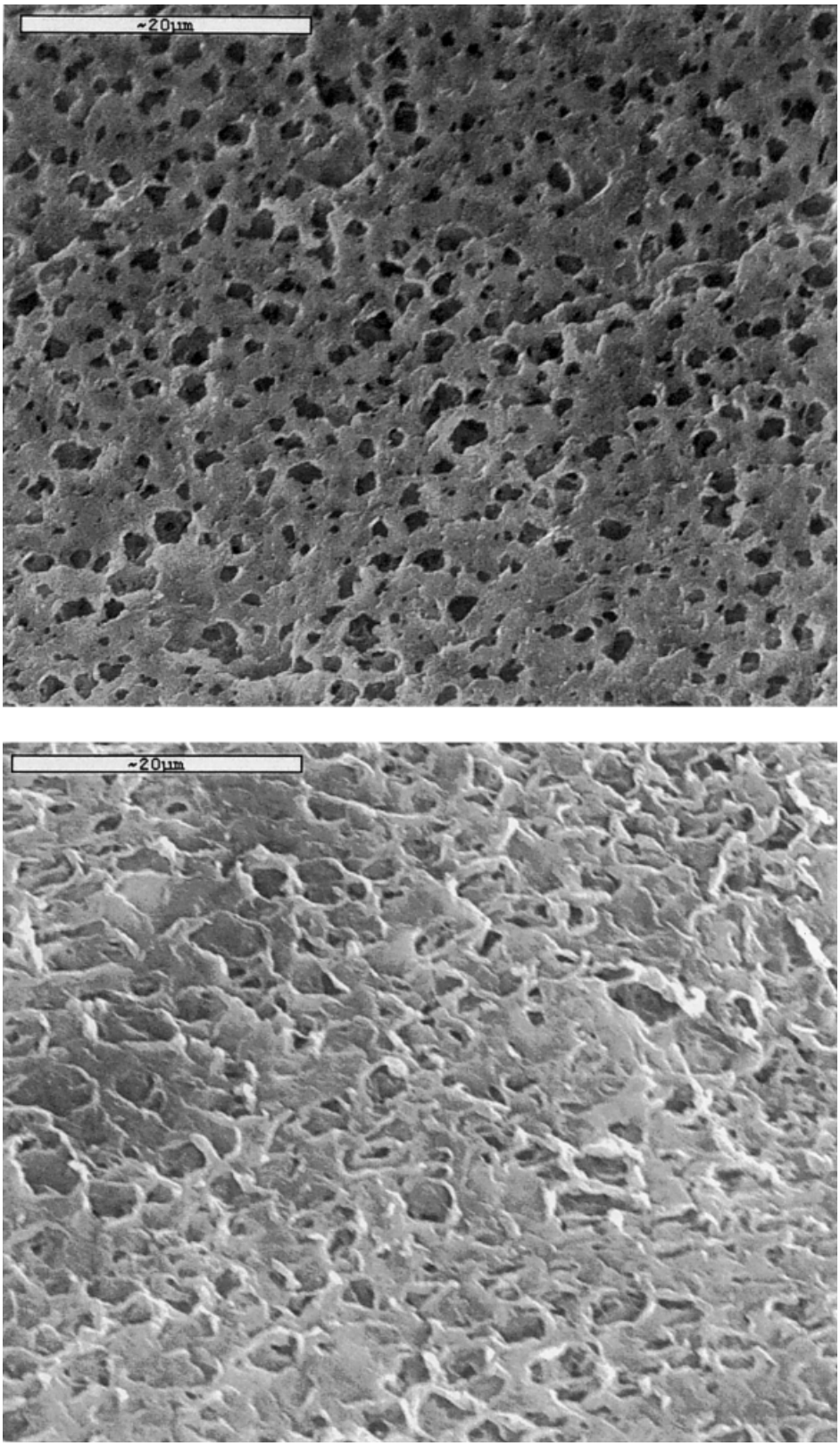

Figure 1 Representative SEM photographs of polyamide hollow fibers: (a) inner surface and (b) outer surface.

meet most of these requirements because they have a narrow pore-size distribution and good mechanical rigidity. The SEM photographs of the surfaces and the cross-section of the polyamide hollow fibers are given in Figure 1. As seen in these photographs, the micropore diameter was around 0.5 μm. The porous struc-

TABLE I
Physical Properties of the Microporous Polyamide Hollow Fibers

Type	PA 386 C
Normal pore size	200 nm
Maximum pore size	430 nm
Wall thickness	110 nm
BET surface area	16 m^2/g
Flux	13 ml/min bar cm^2

ture can play an important role in reducing the diffusional resistance, facilitating mass transfer, and maintaining mechanical strength. This can also provide higher metal chelation and enhance HSA adsorption capacity. Characteristic structural data of the commercially available polyamide hollow fibers were given in Table I.

According to the mercury porosimetry data, the pore radii of the polyamide hollow fibers changed between 200 and 430 nm. This indicated that the hollow fibers contained macropores. This pore diameter range was possibly available for diffusion of the metal ions. Ionic diameters were 0.148 nm for Co^{2+}, 0.114 nm for Cu^{2+}, and 0.138 nm for Ni^{2+}. The molecular dimension of the ellipsoid albumin molecule was 4.0 $\times$ 4.0 $\times$ 14.0 nm (diameter based on sphere $\times$ 5.38 nm). On the basis of these data, the commercially available microporous polyamide hollow fibers had effective pore structures for metal chelation and HSA binding.

The specific surface area of the hollow fiber was found to be 16.0 m^2/g of polymer by the BET method after Cibacron Blue F3GA attachment. Therefore, we concluded that these pores were not blocked by the immobilized dye molecules. This is an important advantage of these modified hollow fibers for further dynamic protein adsorption studies. Polyamide hollow fibers also have several disadvantages. They have a low concentration of primary amino groups, leading to low ligand density and a high nonspecific adsorption of protein due to the hydrophobic nature of polymer surface. These problems can be solved by hydration of the hollow fibers to increase the number of primary amino groups on the matrix binding with polyhydroxyl-containing material such as polyglucose, polyvinyl alcohol, dextran, starch, and cellulose to increase reactive sites and reduce nonspecific adsorption.

Cibacron Blue F3GA is a monochlorotriazine dye (Fig. 2), has been widely immobilized onto various adsorbents, and is used for the purification of both nicotinamid adenine dinucleotide (NAD^+)- and nicotinamid adenine dinucleotide phosphate ($NADP^+$)-dependent dehydrogenase, kinases, glycolytic enzymes, blood proteins including serum albumin, and a number of other seemingly unrelated proteins.[26,27] It contains three sulfonic acid groups and four basic primary and secondary amino groups. The possible active sites to react with proteins are SO_3, NH_2, and NH. The strong binding of the dye ligand to protein may have resulted from the cooperative effect of different mechanisms such as hydrophobic and/or ion-exchange interactions caused by the aromatic structure and acidic groups on the dye ligand and by groups on the side chains of amino acids on the protein molecules. The dye ligands were not very hydrophobic overall, but they did have planar surfaces, which prefer to interact with hydrophobic residues on the protein. On the other hand the chelation of Co^{2+}, Cu^{2+}, and Ni^{2+} ions to the Cibacron Blue F3GA molecules occurred specially through oxygen, nitrogen, and sulfur atoms. Intermediate metal ions (Co^{2+}, Cu^{2+}, Co^{2+}, and Ni^{2+}) coordinate nitrogen, oxygen, and sulfur. When the limited number of free cysteine residues on the surfaces of proteins is considered, this leaves histidine residues as the major targets for intermediate metal ions.[28]

In this study, Cibacron Blue F3GA was immobilized on polyamide hollow fiber, via the reaction between the chlorine groups of the reactive dyes and the primary amino groups of the polyamide hollow fiber. The surface density of Cibacron Blue F3GA obtained on the matrix after hollow-fiber hydration with HCl was 42.5 μmol/g. The studies of Cibacron Blue F3GA leakage from the polyamide hollow fibers showed that there was no leakage in any medium used through out this study, even in a long period of time (more than 52 weeks), which assured us that the washing procedure was quite sufficient for the removal of noncovalently bound Cibacron Blue F3GA molecules from the polymeric matrix.

HSA adsorption from aqueous solution

Effects of pH

Figure 3 shows the effect of pH on the adsorption of HSA with Cibacron Blue F3GA-attached and metal-chelated polyamide hollow fibers. In all the investigated cases, the maximum adsorption of HSA was observed at pH 7.0; with an increase in pH above 7.0 or a decrease in pH under 7.0, the protein adsorption capacity decreased. In general, proteins showed a maximum adsorption efficiency at their isoelectric

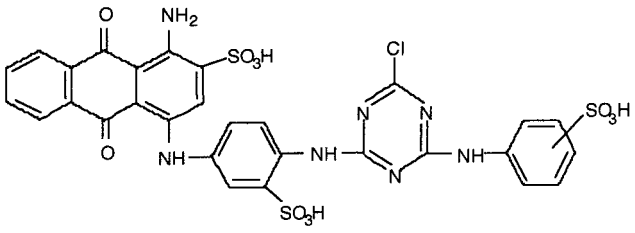

Figure 2 Chemical structure of Cibacron Blue F3GA.

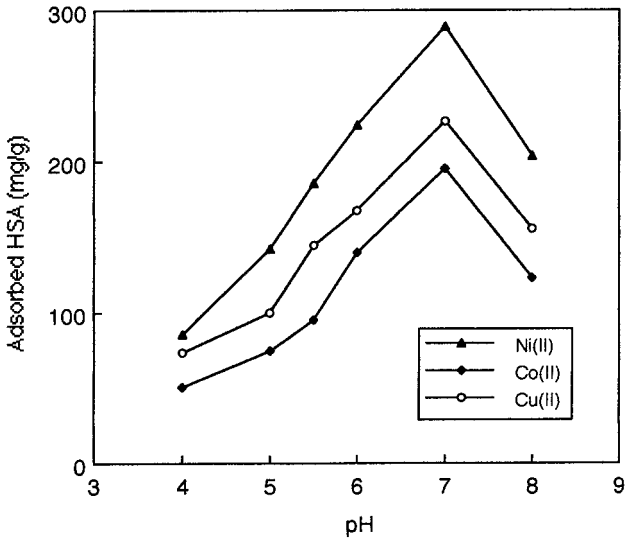

Figure 3 Effect of pH on the HSA adsorption on metal-chelated polyamide hollow fibers. Cibacron Blue F3GA loading = 42.5 μmol/g; metal loading = 150 μmol/g for Cu^{2+} and 250 μmol/g for both Co^{2+} and Ni^{2+}; HSA initial concentration = 7.0 mg/mL; temperature = 25°C.

points because at these points, proteins had no net charge, and therefore, the electrostatic repulsion was diminished. In this study, the maximum adsorption of HSA was shifted from the isoelectric point (pH 5.0) to pH 7.0. This behavior of HSA could be attributed to histidine's pKa of 6.5 because histidines on the HSA surface were largely unprotonated and free to coordinate to the metal ions at pH's of 6.5–7.0, but at alkaline pH, coordination with amino and hydroxyl groups took place and resulted in the less effective adsorption of HSA. This could have also been created from the ionization state of HSA and could have been caused by repulsive electrostatic forces between HSA and the coordinated metal ions. An increase in conformational size of HSA and the lateral electrostatic repulsions between adjacent adsorbed HSA molecules may have also caused a decrease in adsorption efficiency.

Effect of initial concentration

To compare the equilibrium adsorption curves of the three different metal-ion-chelated affinity hollow fibers with HSA, we carried out a series of adsorption experiments at pH 7.0 and 25°C. The equilibrium adsorption curves for the binding of HSA to Co^{2+}, Cu^{2+}, and Ni^{2+} ions chelated onto polyamide hollow fibers are shown in Figure 4. In all the investigated cases, the increases in HSA initial concentration in the adsorption medium led to increases in the adsorbed HSA on the affinity hollow fibers. This was almost linear up to 4.0 mg/mL of HSA and it became constant when the HSA concentration was greater than 4.0 mg/mL. There was, thus, a level in the maximum adsorption capacity for the adsorbents. This could be considered as a typical example of the occupation of all binding sites on the adsorbent surface that were available for HSA adsorption.

A negligible amount of HSA (6.0 mg/g) on the plain matrix was nonspecifically adsorbed. Dye immobilization increased the HSA adsorption capacity of the matrix up to 147 mg/g. Chelation of metals ions onto dye-attached matrix led to a further increase in the adsorption capacity of the affinity matrix to HSA. Maximum HSA adsorption capacities were 195, 226, and 289 mg/g for Co^{2+}-, Cu^{2+}-, and Ni^{2+}-chelated hollow fibers compared to those of the dye-attached hollow fibers, respectively. The affinity of HSA toward the immobilized metals followed the order: Ni(II) > Cu(II) > Co(II).

HSA adsorption from human serum

The adsorption of HSA from human serum was performed in a batch system. Table II shows the HSA adsorption data. There was a very low nonspecific adsorption of HSA (7.5 mg/g) from human serum on the untreated polyamide hollow fibers, although much higher adsorption values were found to be 218 mg/g from human serum when the Cibacron Blue F3GA-attached hollow fibers were used. The adsorption of HSA onto the metal-chelated hollow fibers was drastically higher than those obtained in the studies in which dye affinity hollow fibers were used (Table III). The high HSA adsorption capacity was due to the metal chelation. HSA adsorption capacity was dem-

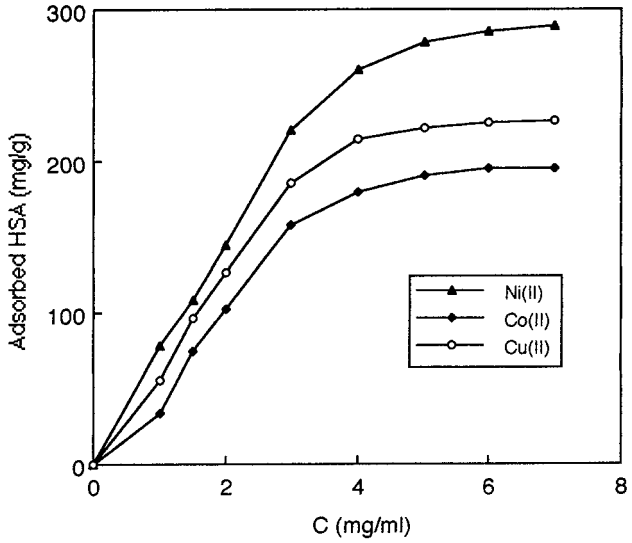

Figure 4 Effect of initial concentration on the HSA adsorption capacity of metal-chelated hollow fibers. Cibacron Blue F3GA loading = 42.5 μmol/g; metal loading = 150 μmol/g for Cu^{2+} and 250 μmol/g for both Co^{2+} and Ni^{2+}; pH = 7.0; temperature = 25°C.

TABLE II
HSA Adsorption from the Serum of a Healthy Donor

HSA concentration (mg/ml)	Amount of HSA adsorbed (mg/g)[a]		
	Co^{2+}	Cu^{2+}	Ni^{2+}
2.5	75	98	125
4.9	98	125	153
9.8	156	178	224
19.6	205	249	295
39.2	245	268	324

Ligand surface concentration; 42.5 μmol/g; T = 25°C; plasma volume = 20 mL.
[a] Each data point is an average of five parallel studies.

onstrated toward the metal ion with the effects in the order: Ni(II) > Cu(II) > Co(II).

Comparison with related literature

Different pseudospecific and metal chelate affinity adsorbents have been used for protein separation from various sources, including human serum. Horstmann et al. used dye-incorporated Sepharose CL-6B, and they reported bovine serum albumin (BSA) adsorption capacities around 5.4–12 mg/g of moist gel.[29] Denizli et al. and his coworkers used different dye and metal chelate polymeric adsorbents including monosize poly(methylmethacrylate-*co*-hydroxy-ethylmethacrylate), poly(vinyl alcohol), and poly(hydroxyethylmethacrylate) microbeads, and they obtained 35–178 mg/g of polymer for HSA.[30–32] Nash and Chase used poly(vinyl alcohol)-modified poly(styrene-diviniyl benzene) microbeads carrying different dye ligands.[33] They presented adsorption capacities of 11.7–27 mg of HSA/g. Boyer and Hsu used Sepharose beads carrying different amounts of Cibacron Blue F3GA (2–25 μmol/mL) and reported adsorption values up to 55.9 mg of BSA/g of polymer.[34] Zeng and Ruckenstein reported 10.2 mg of HSA/g adsorption capacity with Cibacron Blue F3GA-attached polyethersulfone-supported chitosan sorbents.[35] Li and Spencer used Cibacron Blue F3GA-attached polyethylene-imine-coated titania and achieved 4.4 mg of HSA/g.[36] Chase reached 14 mg of BSA/g with Cibacron Blue F3GA-attached Sepharose CL-6B.[37] Tuncel et al. reported 60 mg of BSA/g adsorption capacity with Cibacron Blue F3GA-attached poly(vinyl alcohol)-coated monosize polystyrene microbeads.[38] Muller-Schulte et al. used several polymeric carriers made of different polymers and Cibacron Blue F3GA as the dye ligand.[39] Their albumin adsorption values were in the range of 0.19–0.81 mg of HSA/mL of sorbent. McCreath et al. developed liquid perfluorocarbon supports carrying C.I. Reactive Blue 4, and the maximum capacity of the flocculated emulsion for HSA was found to be 1.81 mg/ml.[40] Odabaşi et al. prepared poly(hydroxyethylmethacrylate methacryloamidohistidine) chelating beads carrying Cu^{2+} ions, and they reported 94.6 mg of HSA/g of polymer adsorption capacity.[41] Adsorption capacities of commercially available crosslinked agarose/Cibacron Blue F3GA sorbents (Bio-Rad, Hercules, CA) were reported as about 11 mg of albumin/mL of sorbent.[42] Comparison of these results shows that metal-chelated hollow fibers exhibited higher albumin adsorption capacities.

Desorption studies and repeated use

The desorption of the adsorbed HSA from the metal-dye chelated hollow fibers was studied in a batch system. The HSA-loaded adsorbents were placed within the desorption medium containing 0.5*M* NaSCN at pH 8.0 or 25 m*M* EDTA at pH 4.9, and the amount of HSA and Co^{2+}, Cu^{2+}, or Ni^{2+} released in 1 h was determined. As seen in Table III, the amount of the adsorbed HSA was desorbed up to 98% in all cases when NaSCN was used as a desorption agent. There was no Co^{2+}, Cu^{2+}, or Ni^{2+} release in this case, which showed that metals ions were chelated to Cibacron Blue F3GA molecules on the matrix surface by strong chelate formation. However, when EDTA was used for desorption, about 36% of HSA was removed from the dye-attached hollow fibers; this may have been because of a salting out effect. Although under the same desorption conditions, about 100% of the HSA were desorbed from the metal-chelated hollow fibers. In this later case, almost all the Co^{2+}, Ni^{2+}, and Cu^{2+} ions initially loaded came out (were released) from the hollow fibers. This means that EDTA broke down the chelates between Co^{2+}, Cu^{2+}, and Ni^{2+} ions

TABLE III
Desorption of HSA and Metal Ions

Reactive ligand	HSA loaded (mg/g)	Elution ratio for HSA (%)		Elution ratio for metal ions (%)	
		NaSCN	EDTA	NaSCN	EDTA
Cibacron Blue F3GA	147	92	36	—	—
Cibacron Blue F3GA/Co^{2+}	195	39	98	0	100
Cibacron Blue F3GA/Cu^{2+}	226	45	99	0	100
Cibacron Blue F3GA/Ni^{2+}	289	43	98	0	100

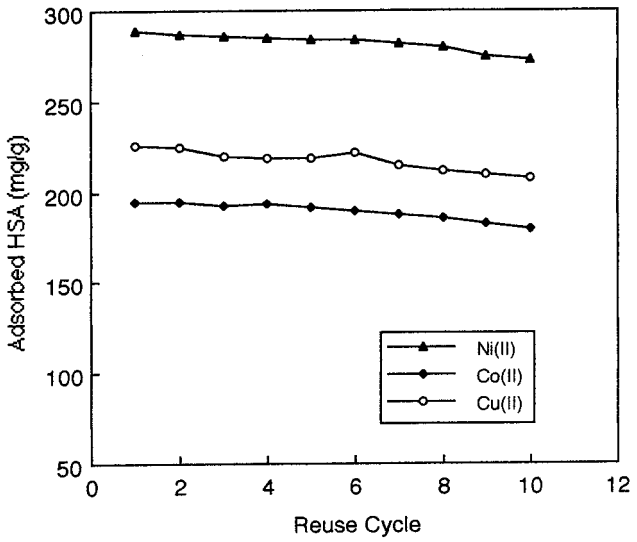

Figure 5 Repeated use of hollow fibers: Cibacron blue loading = 42.5 μmol/g; metal loading = 150 μmol/g for Cu^{2+} ions and 250 μmol/g for both Co^{2+} and Ni^{2+} ions; HSA initial concentration = 7.0 mg/mL; pH = 7.0; temperature = 25°C.

and Cibacron Blue F3GA molecules. With the desorption data given previously, we concluded that NaSCN was a suitable desorption agent, especially for the different metal-chelated hollow fibers, and allowed repeated use of the affinity matrix developed in this study.

To be useful in protein purification processes, adsorbent materials should be easily regenerated under suitable conditions. To show the reusability of the metal-chelated hollow fibers, adsorption–desorption cycle of HSA was repeated 10 times with the same affinity adsorbents. As seen from Figure 5, adsorption capacities for all the adsorbents did not change noticeably during the repeated adsorption–desorption operations. After the 10th run, a decrease of about 7.5% in HSA adsorption capacity was observed, and this value almost remained stable in subsequent runs. These results also showed that metal-chelated hollow fibers were very stable.

CONCLUSIONS

Metal chelate affinity chromatography is a sensitive and selective method for protein separation. The number of locations of surface exposed electron-donating imidazole and thiol groups and their ability to coordinate with chelated metal ions dictate the adsorption of proteins on metal-immobilized adsorbents. Synthetic hollow-fiber adsorbents have several advantageous as support matrices in comparison to conventional bead supports because they are not compressible and they eliminate internal diffusion limitations. Immobilized metal-chelating hollow fibers were investigated for HSA adsorption. Cibacron Blue F3GA loading on polyamide hollow fibers was 42.5 μmol/g. Buffer pH, chelator type, and initial concentration of HSA notably affected HSA adsorption to the adsorbent. The temperature somewhat affected the HSA adsorption. The maximum capacity of dye-attached hollow fibers for HSA adsorption was 147 mg/g. Chelation of Co^{2+}, Cu^{2+}, and Ni^{2+} ions onto the dye-attached hollow fibers led to significant increases in HSA adsorption capacity. This new support had a higher HSA adsorption capacity and showed less nonspecific adsorption. Maximum HSA adsorption capacities were 195 mg/g for Co^{2+}, 226 mg/g for Cu^{2+}, and 289 mg/g for Ni^{2+}. The affinity of HSA toward the immobilized metals followed the order: Ni(II) > Cu(II) > Co(II). Adsorbed HSA molecules were desorbed up to 99% with 0.5*M* NaSCN and 25 m*M* EDTA as the desorption agents. In repeated adsorption/desorption processes, these novel affinity hollow fibers revealed good properties for adsorption of HSA, and they will be effective in processing large volumes of a culture medium containing a target protein.

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