Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Handan Yavuz[a]; Süleyman Patir[b]; Ridvan Say[c]; Yakup Arica[d]; Adil Denizli[a]
[a] Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey [b] Department of Science Education, Hacettepe University, Ankara, Turkey [c] Department of Chemistry, Anadolu University, Eskişehir, Turkey [d] Department of Biology, Kırıkkale University, Kırıkkale, Turkey

Online publication date: 13 June 2002

To cite this Article Yavuz, Handan , Patir, Süleyman , Say, Ridvan , Arica, Yakup and Denizli, Adil(2002) 'Affinity separation of plasma proteins using a newly synthesized methacrylamidoalanine incorporated porous pHEMA membranes', Separation Science and Technology, 37: 9, 2077 — 2095
To link to this Article: DOI: 10.1081/SS-120003502
URL: http://dx.doi.org/10.1081/SS-120003502

SEPARATION SCIENCE AND TECHNOLOGY, 37(9), 2077–2095 (2002)

AFFINITY SEPARATION OF PLASMA PROTEINS USING A NEWLY SYNTHESIZED METHACRYLAMIDOALANINE INCORPORATED POROUS pHEMA MEMBRANES

Handan Yavuz,[1] Süleyman Patir,[2] Ridvan Say,[3] Yakup Arica,[4] and Adil Denizli[1,*]

[1]Department of Chemistry, Biochemistry Division, Hacettepe University, P.K. 51, Samanpazarı, 06242, Ankara, Turkey
[2]Department of Science Education, Hacettepe University, Beytepe, Ankara, Turkey
[3]Department of Chemistry, Anadolu University, Eskişehir, Turkey
[4]Department of Biology, Kırıkkale University, Kırıkkale, Turkey

ABSTRACT

In this study, we synthesized a novel adsorbent to obtain high protein-adsorption capacity utilizing 2-methacrylamidoalanine (MAAL) containing membrane. Amino acid-ligand MAAL was synthesized by using methacrylochloride and alanine. Then, poly(2-hydroxyethylmethacrylate-*co*-2-methacrylamidoalanine)

*Corresponding author. Fax: (90) 312 2992163; E-mail: denizli@hacettepe.edu.tr

[p(HEMA-*co*-MAAL)] membranes were prepared by UV-initiated photopolymerization of HEMA and MAAL in the presence of an initiator (azobisisobutyronitrile, AIBN). Synthesized MAAL was characterized by nuclear magnetic resonance spectroscopy. p(HEMA-*co*-MAAL) membranes were characterized by swelling studies, porosimeter, scanning electron microscopy, Fourier transform-infra red spectroscopy, and elemental analysis. These membranes have macropores in the size range 5–10 μm. Different metal ions including Zn(II), Ni(II), Co(II), and Cu(II) were chelated on these membranes. p(HEMA-*co*-MAAL) were used in the adsorption of human serum albumin (HSA) from aqueous media containing different amounts of albumin (0.1–5.0 mg L^{-1}) and at different pH values (4.0–8.0). The maximum HSA adsorption was observed at pH 5.0. The nonspecific adsorption of HSA on the pHEMA membranes was negligible 0.9 μg cm^{-2}. MAAL incorporation significantly increased the HSA adsorption (1.76 mg cm^{-2}). The HSA adsorption capacities of the metal-incorporated membranes were greater than that of the p(HEMA-*co*-MAAL) membranes under the same conditions. Higher HSA adsorption capacity was observed from the human plasma (2.88 mg HSA cm^{-2}).

Key Words: Albumin adsorption; Amino acid membranes; Poly(HEMA); Metal chelates

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in the circulatory system. It has many important physiological functions, which contribute significantly to colloid osmotic blood pressure and aid in the transport, distribution, and metabolism of many endogeneous and exogeneous substances including bile acids, bilirubin, long-chain fatty acids, amino acids, steroids, metal ions, and numerous pharmaceuticals (1). HSA consists of a single, nonglycosylated, polypeptide chain containing 585 amino acid residues. Its amino acid sequence contains a total of 17 disulfide bridges, one free thiol and a single tryptophan (2). The research on albumin separation has attracted considerable attention due to its great potential in blood protein manufacture. HSA is at present commonly isolated from human plasma by Cohn's classical blood fractionation procedure (3). Cohn's method concerns precipitation of proteins using ethanol with varying pH, ionic strength, and temperature. But this

technique, which is the oldest method of industrial fractionation of blood proteins, is not highly specific and can give partially denaturated proteins (4).

Bioaffinity chromatography is already a well-established method for identification, purification, and separation of biomolecules, and based on highly specific molecular recognition or biorecognition. In this method, the molecule possessing a specific recognition capability (i.e., ligand) is immobilized on a suitable insoluble support, which is usually a polymeric material in bead or membrane form. The molecule to be isolated is selectively captured by the complementary ligand immobilized on the matrix. Bioaffinity chromatography is an alternative technique to Cohn's fractionation. Biological ligand stability is becoming an increasingly important consideration. The trend, therefore, has been to replace high molecular-mass biological ligands with small molecular-mass pseudospecific ligands (5,6). Especially, the small amino acid ligands offer advantages over biological ligands in terms of economy, ease of immobilization, chemical stability, and high adsorption capacity (5–8). The idea of using different amino acids by these researchers, stems from the fact that amino acids are very reactive with different chemical substances including proteins. The higher flexibility and durability of these amino acid ligands as well as significantly lower material and manufacturing costs are also very important. In addition, amino acids may be easily modified by existing chemical methods to facilitate desorption under mild conditions (9).

The bead-packed columns are effective and widely used for purification of biomolecules. Inherent limitations in bead-packed column liquid chromatography include relatively time consuming and high-pressure packing process, high-pressure drop of the columns, the fouling, and the slow diffusion of solutes within the pores of the beads matrix (10). One significant trend has been to replace spherical beads packing by sheets of microporous membranes and macroporous monolithic rods that allow at low pressure (11–15). The desirable properties of affinity membranes are high porosity, large internal surface area, high chemical, biological and mechanical stabilities, hydrophilicity, low nonspecific adsorption, and the presence of functional groups for derivatization (16). Due to these advantages, microporous affinity membranes provide higher efficiency (17).

For these reasons, we have focused our attention on the development of amino acid-incorporated affinity membranes (18). In this approach, the most important advantages are the elimination of chemical activation and ligand immobilization steps. Our purpose of this study was to extend further our earlier attempts to use the pseudospecific adsorption and metal chelate affinity adsorption. This article considers preparation of a porous membrane system containing 2-methacrylo-amidoalanine for albumin adsorption. p(HEMA-*co*-MAAL) membranes were prepared by UV-initiated photopolymerization of HEMA and MAAL monomers. Adsorption conditions (i.e., initial concentration of albumin, medium pH, and temperature) were varied to evaluate their effects on protein adsorption.

EXPERIMENTAL

Materials

Methacrylochloride was purchased from Sigma (St. Louis, MO). The monomer, HEMA, was obtained from Fluka A.G. (Buchs, Switzerland), distilled under reduced pressure in the presence of hydroquinone inhibitor and stored at 4°C until use. Azobisisobutyronitrile (AIBN) was provided from Fluka (Buchs, Switzerland). The HSA was supplied from Sigma Chemical Co. (St. Louis, MO) and used as received. All of the other chemicals used were reagent grade from Merck AG (Darmstadt, Germany). The metal salt used was $Cu(NO_3)_2$. The pH of solutions was adjusted with nitric acid and sodium hydroxide. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion-exchange packed-bed system. The resulting purified water (deionized water) has a specific conductivity of 18 megaohm cm^{-1}. All glassware was soaked overnight in 4 *M* nitric acid.

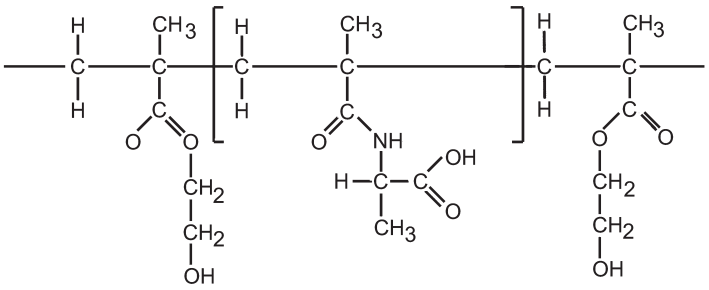
Synthesis of MAAL

For the synthesis of MAAL, the following experimental procedure was applied: 5.0 g of alanine and 0.2 g of $NaNO_2$ were dissolved in 30 mL of K_2CO_3 solution (5%, v/v). This solution was cooled down to 0°C. Six milliliters of methacrylochloride was poured slowly into this solution under nitrogen atmosphere and then stirred magnetically at room temperature for 2 hr. At the end of this chemical reaction period, pH of this solution was adjusted to 3.0. Then solution was extracted with chloroform ($CHCl_3$). The organic phase was dried with $MgSO_4$ and evaporated in rotary evaporator. The residue (i.e., MAAL) was crystallized in ether–cyclohexane mixture.

Preparation of Poly(HEMA-*co*-MAAL) Membranes

The p(HEMA-*co*-MAAL) membrane was prepared as described in the following section. Two milliliters of HEMA containing 5 mg AIBN as polymerization initiator and 100 mg of synthesized MAAL was mixed with 3 mL of 0.1 *M* $SnCl_4$. The mixture was then poured into a round glass mold (9 cm in diameter) and exposed to ultraviolet radiation for 10 min under nitrogen atmosphere. The membrane obtained was washed several times with distilled water, and cut into circular pieces (1.0 cm in diameter) using a perforator.

Polymer chemical structure, which was produced with HEMA and MAAL monomers, is given as follows:



Characterization of Poly(2-HEMA-*co*-2-MAAL) Membranes

Swelling Test

Swelling ratios of the pHEMA and p(HEMA-*co*-MAAL) membranes were determined in distilled water. The experiment was conducted as follows: initially dry membrane sample with a fixed surface area and thickness (ϕ: 1 cm; thickness: 800 μm) were carefully weighed before being placed in a 50 mL vial containing distilled water. The vial was put into an isothermal water bath with a fixed temperature (25 $\pm$ 0.5°C) for 2 hr. The membrane sample was taken out from the water periodically, wiped using a filter paper, and weighed. The weight ratio of dry and wet samples was recorded.

Microscopic Observations

Surface morphology and internal structure of p(HEMA-*co*-MAAL) membranes were observed in a scanning electron microscope (SEM) (JEOL, JEM 1200EX, Tokyo, Japan). p(HEMA-*co*-MAAL) membranes 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 $\times$ 1000 magnification.

Porosity Measurements

Pore volumes and average pore diameter greater than 20 Å were determined by mercury porosimeter up to 2000 kg cm^{-2} using a Carlo Erba model 200 (Milan, Italy).

Elemental Analysis

To evaluate the degree of MAAL incorporation, the synthesized p(HEMA-*co*-MAAL) adsorbents were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932, Chicago, IL).

FTIR Studies

FTIR spectra of the pHEMA and p(HEMA-*co*-MAAL) membrane were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Tokyo, Japan). The dry membrane (about 0.1 g) was thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Darmstadt, Germany), and pressed into a form a tablet, and the FTIR spectrum was then recorded.

NMR Studies

The proton NMR spectrum of MAAL monomer was taken in $CDCl_3$ on a JEOL GX-400 300 MHz instrument. The residual nondeuterated solvent ($CHCl_3$) served as an internal reference. Chemical shifts are reported in ppm (d) downfield relative to $CHCl_3$.

Chelation of Metal Ions

Chelation of metal ions including Zn(II), Ni(II), Co(II), and Cu(II) from the single metal ions aqueous solutions was investigated in batch adsorption-equilibrium experiments. Twenty milliliters of aqueous metal ion solution was treated with the pHEMA and p(HEMA-*co*-MAAL) membranes for 2 hr (equilibrium adsorption time). The flask was magnetically stirred at an agitation speed of 100 rpm at room temperature. Initial concentration of metal ion was 10 ppm. Medium pH was 4.0. Nitrate salts were used for preparing standard metal ion solutions.

HSA Adsorption from Aqueous Solutions

In these experiments, the effects of initial protein concentration, medium pH, and ionic strength on the adsorption capacity of p(HEMA-*co*-MAAL) membranes 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 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). Ionic strength of the adsorption media was changed in the range of 0.05–0.5. HSA concentration was varied between 1.0 and 7.0 mg mL^{-1}. In a typical adsorption experiment, HSA was dissolved in 10 mL of buffer solution, and five pieces of membranes (ϕ: 1.0 cm) were added. Then the adsorption experiments were conducted for 2 hr (equilibrium time) at 4°C at a stirring rate of 100 rpm. At the end of this equilibrium period, HSA adsorption by the p(HEMA-*co*-MAAL) membranes was determined by measuring the initial and final concentrations of HSA within the adsorption medium using Coomassie Brilliant Blue as described by Bradford (19).

HSA Adsorption from Human Plasma

HSA adsorption from human plasma with p(HEMA-*co*-MAAL) membranes 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 41.0 mg HSA mL^{-1} as determined by bromocresol green (BCG) dye method at 628 nm (20). Ten milliliters of the freshly separated human plasma was incubated with membrane pieces pre-equilibrated with acetate buffer (pH 5.0) for 2 hr. These experiments were conducted at 4°C and a stirring rate of 100 rpm. The amount of HSA adsorbed by p(HEMA-*co*-MAAL) membranes was determined by measuring the initial and final concentrations of HSA in plasma. Phosphate buffered saline (PBS, pH: 7.4, NaCl: 0.9%) was used for dilution of human plasma.

In order to show MAAL specificity, competitive blood protein adsorption (i.e., albumin, fibrinogen, and immunoglobulin-G) was also studied. The p(HEMA-*co*-MAAL) membranes were incubated with a human plasma containing albumin (41.0 mg mL^{-1}), fibrinogen (2.4 mg mL^{-1}), and γ-globulin (18.2 mg mL^{-1}) at room temperature for 2 hr. Total protein concentration was measured using the total protein reagent (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK; Catalog Ref. No: 712076) at 540 nm, which is based on Biuret reaction (20). Chronometric determination of fibrinogen according to the Clauss method on plasma was performed using a Fibrinogene-Kit (Ref. No.: 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France) (21). The HSA concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK; Catalog Ref. No.: 229241), which is based on bromocresol green (BCG) dye method (20). γ-globulin concentration was determined from the difference.

RESULTS AND DISCUSSION

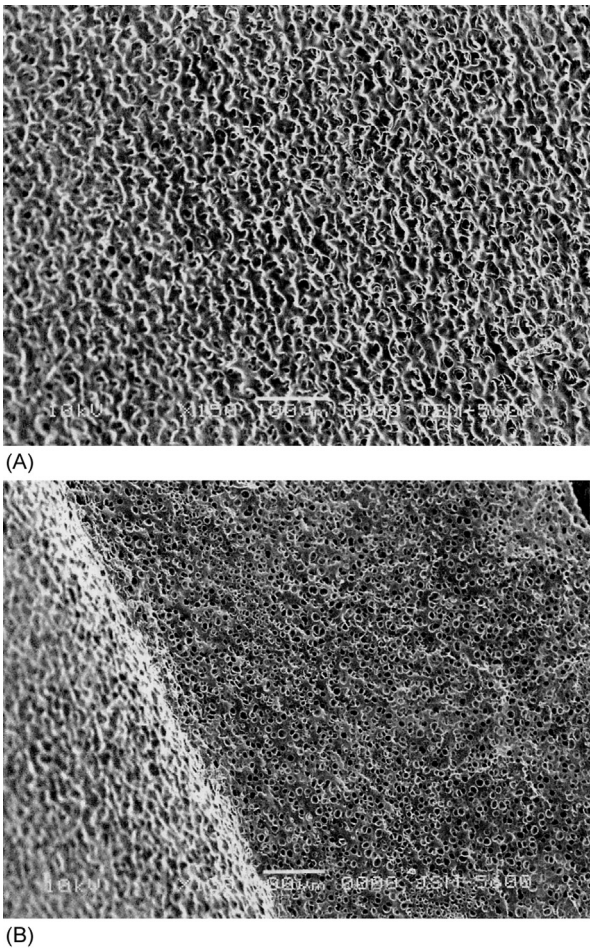
Properties of Poly(HEMA-*co*-2-MAAL) Membranes

Both the pHEMA and the p(HEMA-*co*-MAAL) membranes prepared in this study are rather hydrophilic structures. Therefore, they do swell in aqueous solutions, but do not dissolve. The equilibrium water-uptake is reached in about 60 min. Compared with pHEMA (58%), the water swelling ratio of the p(HEMA-*co*-MAAL) membrane increases significantly (199.9%). Several possible factors may contribute to this result. First, incorporating MAAL actually introduces more hydrophilic functional groups into the polymer chain, which can attract more water molecules into polymer matrices. Second, reacting MAAL with HEMA could effectively decrease the molecular weight of resulting polymer and reduce the crystallinity of polymeric structure. Therefore, the water molecules penetrate into the polymer chains more easily, resulting in an improvement of polymer water swelling in aqueous solutions.

According to the mercury porosimetry data, the pore radii of the p(HEMA-*co*-MAAL) changed between 500 and 800 nm. This indicated that the p(HEMA-*co*-MAAL) membranes contained mainly macropores. This pore diameter range is possibly available for diffusion of the metal ions. It should be noted that ionic diameter of metal ions is around 0.2 nm. Molecular diameter of albumin is also 83.5 Å (molecular weight of albumin: M_w: 67,000). On the basis of these data, it was concluded that the p(HEMA-*co*-MAAL) membranes have effective pore dimensions for both metal chelation and protein adsorption.

The SEM micrographs given in Fig. 1 show the surface structure and the cross-section of the p(HEMA-*co*-MAAL) membranes. As seen from the surface and cross-sectional surface photographs, the membranes have large pores (transport canals); the micropore dimensions are around in the range $5-10\,\mu$m. The membrane surface seems to be rough and heterogeneous. These large pores reduce diffusional resistance and facilitate mass transfer of heavy-metal ions because of high inner-surface area. This also provides higher heavy-metal chelation and enhances adsorption capacity.

As mentioned before, MAAL was selected as the metal-complexing ligand. In the first step, MAAL was synthesized from alanine and methacrylochloride. Then, MAAL was incorporated into the bulk structure of the pHEMA membranes. The FTIR spectra of pHEMA and p(HEMA-*co*-MAAL) have the characteristic stretching vibration band of hydrogen-bonded alcohol, O—H, around 3440 cm^{-1} (Fig. 2). The FTIR spectrum of p(HEMA-*co*-MAAL) have characteristic amide I and amide II absorption bands at 1660 and 1520 cm^{-1}, respectively. On the other hand, hydrogen-bonded alcohol



(A)

(B)

Figure 1. The electron micrographs showing (A) surface; (B) cross-sectional area of p(HEMA-*co*-MAAL) membranes (magnification: × 1000).

O—H stretching band intensity of plain pHEMA is higher than that of p(HEMA-*co*-MAAL) membrane due to the incorporation of MAAL comonomer in the polymer structure.

To evaluate the degree of MAAL incorporation, elemental analysis of the synthesized p(HEMA-*co*-MAAL) adsorbent was performed. The incorporation of the MAAL was calculated as 23.9 mmol MAAL m^{-2} based on theoretical amount of nitrogen contributed by the monomer mixture.

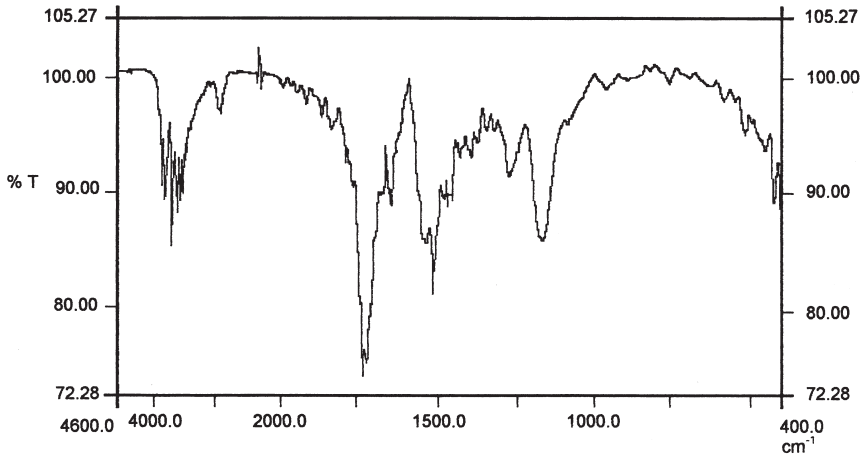


Figure 2. The FTIR spectrum of p(HEMA-*co*-MAAL) membranes.

^{1}H-NMR was used to determine the synthesis of MAAL structure. The ^{1}H-NMR spectrum is shown to indicate the characteristic peaks from the groups in MAAL monomer (Fig. 3). These characteristic peaks are as follows: ^{1}H-NMR ($CDCl_3$): δ 1.51 (δ; 3H, $J = 7.1$ Hz, CH_3), 1.99 (s; 3H), 4.65 (t; 1H, methin, $J = 7.1$ Hz), 5.42 (s; 1H, vinyl), 5.8 (s; 1H, vinyl); 6.52 (δ; 1H, amide, $J = 6.2$ Hz),10.18 (bs; 1H).

Albumin Adsorption from Aqueous Solution

Effect of Initial Concentration of Albumin

Figure 4 shows the effect of initial albumin concentration on adsorption. As shown in this figure, with increasing HSA concentration in solution, the amount per unit area of albumin adsorbed by the membrane increases almost linearly at low concentrations, below about 3.0 mg mL^{-1}, then increases less rapidly and approaches saturation, which may be considered as a typical example of the occupation of all of the active surface groups on the adsorbent surface that are available for HSA molecules. The steep slope of the initial part of the adsorption isotherm represents a high affinity between albumin and MAAL molecules. It becomes constant when the protein concentration is greater than 3.0 mg mL^{-1}. Negligible amount of albumin adsorbed nonspecifically on the pHEMA membrane is 0.9 μg cm^{-2}. MAAL

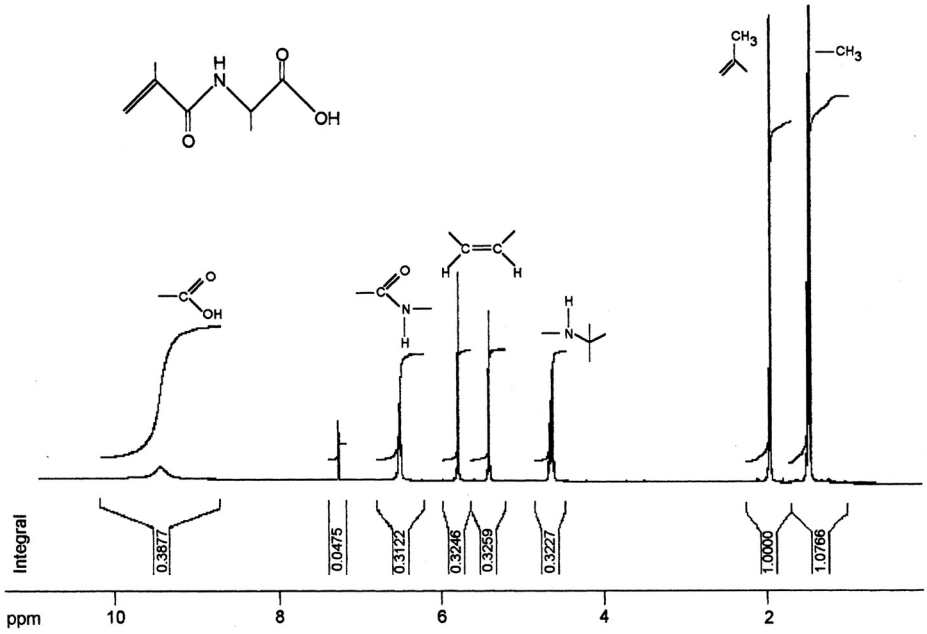


Figure 3. The NMR spectrum of MAAL monomer.

incorporation increased the HSA adsorption capacity of the membranes up to 1.76 mg cm^{-2}. It is clear that this increase in adsorption capacity is due to weak forces (i.e., secondary interactions such as hydrogen bonding, electrostatic and hydrophobic interactions, and van der Waals forces) between MAAL groups and albumin molecules. Nevertheless, these MAAL-containing membranes exhibit a high selectivity resulting from the cumulative effects of multiple weak binding events with fast kinetics.

Note that a wide variety of sorbents with a wide range of adsorption capacities were reported in literature for albumin adsorption. Denizli et al. found 4.1 μg cm^{-2} adsorption capacity with dye-ligand Congo Red attached monosize poly(methylmethacrylate-2-hydroxyethylmethacrylate) microspheres (22). Tuncel et al. reached adsorption capacity of 4.0 μg BSA cm^{-2} with the polylvinylalcohol-coated Cibacron Blue F3GA immobilized monosize polystyrene microspheres (23). Zeng and Ruckenstein reported 13.9 μg HSA cm^{-2} with the Cibacron Blue F3GA-attached microporous chitosan membranes (24). Denizli et al. showed 26.4 μg BSA cm^{-2} with the poly(vinyl alcohol) particles containing Cibacron Blue F3GA (25). Li and Spencer presented adsorption capacity of 40.0 μg HSA cm^{-2} with Cibacron Blue F3GA attached poly(ethylene

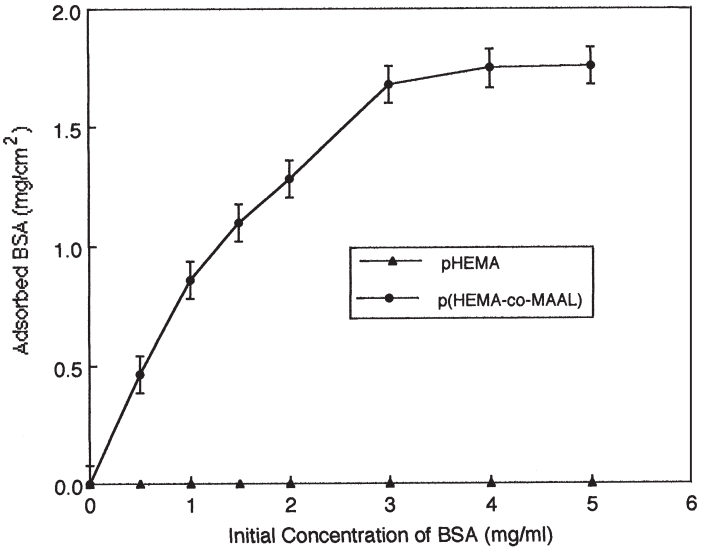


Figure 4. Effect of HSA initial concentration on HSA adsorption; MAAL loading: 23.9 mmol m^{-2}; pH: 5.0; *T*: 25°C; total membrane surface area in each batch: 100 cm^2 L^{-1}. Each data is average of five parallel studies.

imine)-coated titania (26). Ratnayake and Regnier reported very low protein adsorption capacities between 0.122 and 0.216 μg cm^{-2} with a carboxylic acid-attached polyacrylate cation-exhange sorbents (27). Kang et al. immobilized 6.25 μg albumin cm^{-2} on the polymethylmethacrylate surface (28). The maximum HSA adsorption that we achieved with the novel adsorbent system developed in this study was 1.76–2.88 mg cm^{-2} membrane, which was quite high with the related literature.

Effect of pH

The amount of albumin adsorbed onto the p(HEMA-*co*-MAAL) membranes as a function of pH exhibits two adsorption domains, as shown in Fig. 5. It should be noted that the amount of HSA adsorbed onto both p(HEMA-*co*-MAAL) membranes shows a maximum at pH 5.0, with a very significant decrease at lower and higher pH values (isoelectric point of albumin: 5.0). In the case of the use of p(HEMA-*co*-MAAL) membranes, hydrophobic interactions and hydrogen bonding between HSA and MAAL at pH 5.0 may be resulted from the amino acid side chains in HSA, and from

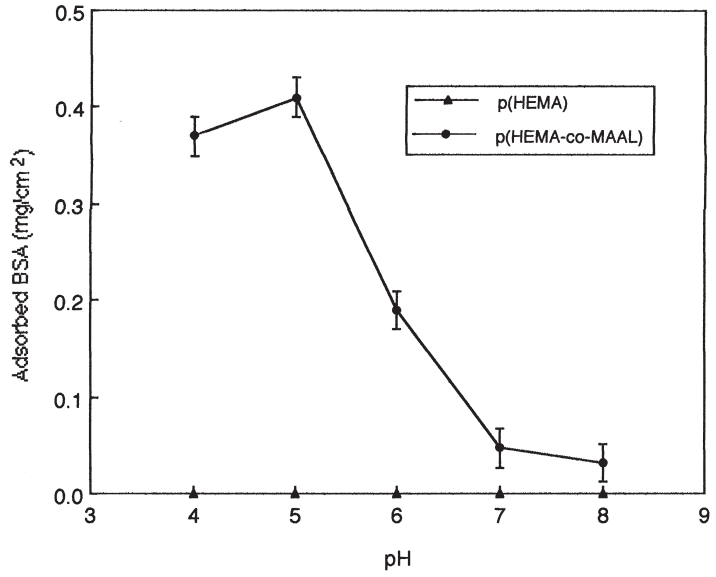


Figure 5. Effects of pH on albumin adsorption; initial concentration of MAAL loading: 23.9 mmol m^{-2}; albumin concentration: 0.5 mg mL^{-1}; *T*: 25°C; total membrane surface area in each batch: 100 cm^2 L^{-1}. Each data is average of five parallel studies.

the conformational state of HSA molecules (more folded structure) at this pH. At pH values lower and higher than pH 5.0, the adsorbed amount of HSA drastically decreases. This could be created from the ionization state of HSA and could be caused repulsive electrostatic forces between HSA and the amino-ligand molecules. Increase in conformational size and the lateral electrostatic repulsions between adjacent adsorbed HSA molecules may also cause a decrease in the protein adsorption efficiency.

Effect of Ionic Strength

The effect of ionic strength (adjusted by adding NaCl) on HSA adsorption is presented in Fig. 6, which shows that the adsorption capacity decreases with increasing ionic strength of the binding buffer (acetate buffer, pH: 5.0) above 0.01 *M* NaCl concentration. The total adsorption capacity of HSA decreases by about 34% as the NaCl concentration changes from 0.01 to 0.1 *M*. The decrease in the adsorption capacity as the ionic strength increases can be attributed to the repulsive electrostatic interactions between the p(HEMA-*co*-MAAL) membranes

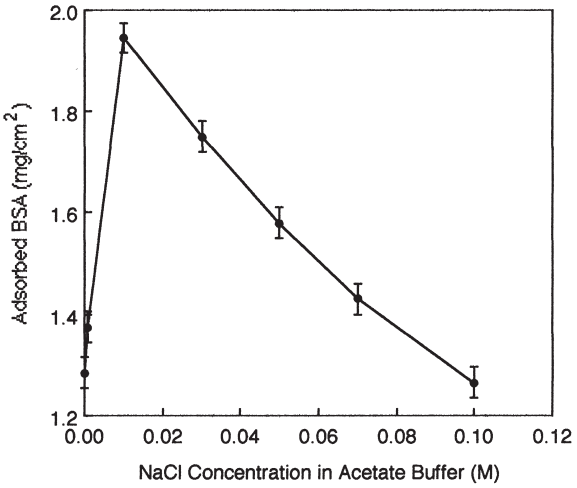


Figure 6. Effect of the ionic strength on HSA adsorption on the p(HEMA-*co*-MAAL) membranes; MAAL loading: 23.9 mmol m^{-2}; initial HSA concentration: 2.0 mg mL^{-1}; pH: 5.0, and *T*: 25°C. Each data is average of five parallel studies.

and HSA molecules. When the salt concentration increases in the adsorption medium, this can lead to coordination of the deprotonated carboxylic acid groups of the MAAL with sodium ions of the salt (NaCl), which leads to low protein adsorption. The distortion of existing salt bridges in the presence of salt also contributed to this low protein adsorption at high ionic strength.

Effect of Metal Type

Although different mechanisms of metal affinity interactions with proteins have been proposed (29), 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 mode. However, more than one type of interaction mechanism is operational (30). The major functional groups on protein contributing toward the interaction with immobilized metal ion consist of the histidine residue and the sulfur atom of the sulfidryl group of the free cysteine residue. While maintaining a free cysteine residue in a natural protein is rare (31), the exposed histidine residue is the dominant binding site in protein adsorption with an immobilized metal ion (32). Factors influencing the interactions include the number of electron-donating

groups on the protein surface, medium pH, concentration of protein, type of metal ions, ligand density, type, and size of chelating ligand.

MAAL contains one carboxylic acid group and one primary amino groups. Theoretically, one expects that one donor atom (i.e., nitrogen, sulfur or oxygen) coordinated with one metal ion giving different geometrical structures such as planar, tetrahedral or octahedral arrangements around the metal ion (coordination number of metal ions studied here is four). Other free coordination valencies of the metal ions are occupied by water molecules. Figure 7 shows the effects of metal ion incorporation on the HSA adsorption. HSA adsorption capacity on naked membranes was 1.1 mg cm^{-2}. HSA adsorption capacities on metal-chelated membranes were found to be 1.77 mg cm^{-2} for Zn(II), 1.69 mg cm^{-2} for Cu(II), 1.61 mg cm^{-2} for Co(II) and 1.23 mg cm^{-2} for Ni(II) ions under the same conditions. We have shown that the metal ion incorporation significantly increased the HSA adsorption capacity of the membranes. This is due to the preferential interaction between HSA molecules (especially imidazole side chains of histidine residue in albumin structure) and chelated metal ions. HSA adsorption capacity was demonstrated towards the metal ion with the effects in the order: Zn(II) > Cu(II) > Co(II) > Ni(II).

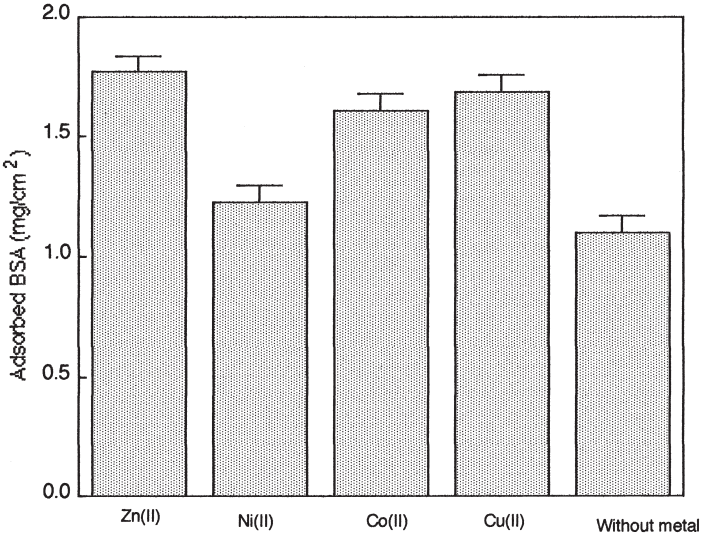


Figure 7. Effect of metal ion type on HSA adsorption on the p(HEMA-*co*-MAAL) membranes; MAAL loading: 23.9 mmol m^{-2}; initial metal concentration: 10 ppm; metal loading pH: 4.0; initial HSA concentration: 1.5 mg mL^{-1}; pH: 5.0, and *T*: 25°C. Each data is average of five parallel studies.

HSA Adsorption from Human Plasma

The adsorption of HSA from human plasma was performed batch system. Table 1 shows the adsorption for human serum obtained from a healthy donor. There was a very low nonspecific adsorption of HSA (1.6 $\mu g\,cm^{-2}$) on the pHEMA membranes, while much higher adsorption values (2.88 $mg\,cm^{-2}$) were obtained when the p(HEMA-*co*-MAAL) membranes were used. It is worth noting that adsorption of HSA onto the p(HEMA-*co*-MAAL) membranes was approximately 1.63-fold higher than those obtained in the studies in which aqueous protein solutions were used. This may be explained as follows; the conformational structure of HSA molecule within their native environment (i.e., human plasma) much more suitable for specific interaction with the p(HEMA-*co*-MAAL) membrane. The high HSA concentration (41.0 $mg\,mL^{-1}$) 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., p(HEMA-*co*-MAAL) membrane].

In order to show the MAAL specificity, competitive protein adsorption was also studied. Interesting results were obtained in these studies. Adsorption capacities were obtained as 2.88 $mg\,cm^{-2}$ for albumin, 0.45 $mg\,cm^{-2}$ for fibrinogen and 0.23 $mg\,cm^{-2}$ for γ-globulin. The total protein adsorption was determined as 3.62 $mg\,cm^{-2}$. It is worth noting that adsorption of other plasma proteins (i.e., fibrinogen and γ-globulin) on the p(HEMA-*co*-MAAL) membranes are not high. 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 1. The HSA Adsorption from the Plasma of a Healthy Donor; MAAL Loading: 23.9 $mmol\,m^{-2}$; *T*: 25°C; Plasma Volume: 4.0 mL

HSA Concentration ($mg\,mL^{-1}$)	Amount of HSA Adsorbed[a] ($mg\,cm^{-2}$)
5.2	0.73 ± 0.18
10.3	1.21 ± 0.20
20.5	1.45 ± 0.22
30.8	2.21 ± 0.28
41.0	2.88 ± 0.25

[a] Each data is average of five parallel studies.

CONCLUSIONS

Bioaffinity chromatography is one of the most efficient technique for the direct separation, isolation, and purification of biological molecules from complex mixtures. Small ligands consisting of a few amino acids may hold certain advantages for industrial affinity bioseparations since they are not likely to cause an immune response in case of leakage into the product (6–9). Small pseudospecific amino acid ligands are also much more stable than biospecific ligands including proteins and antibodies because they do not require a specific tertiary structure for maintaining biological activity. The low cost of amino acid ligands and the ease of regeneration of the polymer-based adsorbents are the other attractive features of pseudospecific affinity separation. We have demonstrated in this article that p(HEMA-*co*-MAAL) pesudospecific microporous affinity membranes are effective in the separation of large amount of albumin from human plasma. These first attempts to use the MAAL containing pHEMA membranes for the routine isolation of albumin from human plasma have yielded promising results.

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Received March 2001
Revised September 2001