# Antibody Purification from Human Plasma by Metal-Chelated Affinity Membranes

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**ABSTRACT:** The aim of this study is to investigate in detail the feasibility of poly(2-hydroxyethyl methacrylate-*N*-methacryloyl-(L)-histidine methyl ester), PHEMAH membranes for purification of immunoglobulin G (IgG) from human plasma. PHEMAH membranes were prepared by photo-polymerization technique. Then, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Cu^{2+}$ ions were chelated directly on the PHEMAH membranes. Elemental analysis assay was performed to determine the nitrogen content and polymerized MAH was calculated as 168.5 μmol/g. The nonspecific IgG adsorption onto the plain PHEMA membranes was negligible (about 0.25 mg/mL). A remarkable increase in the IgG adsorption capacities were achieved from human plasma with PHEMAH membranes (up to 68.4 mg/mL). Further increase was observed with the metal-chelated PHEMAH membranes (up to 118 mg/mL). The metal-chelate affinity membranes allowed the one-step separation of IgG from human plasma. The binding range of metal ions for surface histidines from human plasma followed the order: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$. Adsorbed IgG was eluted using 250 m*M* EDTA with a purity of 94.1%. IgG molecules could be repeatedly adsorbed and eluted with the metal-chelated PHEMAH membranes without noticeable loss in their IgG adsorption capacity. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 3476–3484, 2012

**Key words:** IMAC; affinity membranes; Immunoglobulin-G; PHEMA

## INTRODUCTION

Immunoglobulins represent glycoproteins having carbohydrate-recognition motifs and are widely used for immunodiagnostics, epitope mapping, and therapeutic applications.[1] Several chromatographic techniques including high performance, size exclusion, ion-exchange, hydrophobic interaction, hydroxyapatite, and thiophilic have been used for the purification of immunoglobulins.[2–5] However, immunoglobulins are often purified by protein A affinity chromatography due to its high selectivity.[6,7] Protein A binds with different affinity to the Fc region of immunoglobulins from a variety of sources, e.g., it binds IgG molecules from human, rabbit, and pig with high affinity, binds horse and cow IgG with lower affinity and binds rat IgG only a very weakly.[8] It exhibits a very high specificity and can therefore be employed as a one-step procedure for the purification of immunoglobulins.[9] But, protein A-based adsorbents are expensive and difficult to handle, sterilize, and preserve.[10]

Immobilized metal ion affinity chromatography (IMAC) has been used for purification of proteins.[11–14] IMAC introduces a new approach for selectively interacting biomolecules on the basis of their affinities for metal ions. The separation is based on different binding abilities of the proteins to the chelated metal ions on support. The dominating electron-donating group in a protein is the imidazole side chain of histidine, whereas the *N*-terminus of the protein contributes to a lesser extent. In addition, the thiol group of cysteine would be a good electron donor, but it is rarely present in the appropriate, reduced state.[15] The number of histidine residues in the protein is of primary importance in the overall affinity for immobilized metal ions. In addition, factors such as the accessibility, microenvironment of the binding residue, co-operation between neighboring amino acid side chains, and local conformations play important roles in biomolecule adsorption.[16] Tryptophan also has some contributions.[17] The low cost of metals and the ease of regeneration of the supports are the attractive features of metal affinity separation.

In this study, *N*-methacryloyl-(L)-histidine methyl ester (MAH) was employed as a metal-complexing ligand for use in the IMAC for IgG. Imidazole of MAH has a chelating property with transition metal ions. The purification of IgG is generally required for the purposes of immunodiagnostics and immunotherapy. Moreover, IgG removal from human plasma is employed for the treatment of immune disorders, alloimmunization, and cancer.[18–21] For this reason,

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clinical applications of polymeric carriers including macroporous cryogels and membranes could be a potentially attractive tool.[22–26] Poly(hydroxyethyl methacrylate) [PHEMA] based membranes, which is widely used for biomedical applications due to their biocompatibility and stability, were produced by photo-polymerization.[27,28] Then, $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Cu^{2+}$ ions were chelated. IgG adsorption on the metal-chelated membranes from human plasma is reported here.

## EXPERIMENTAL

### Materials

Human immunoglobulin-G (lyophilized, Cat. No: 160101), L-histidine methyl ester (98% pure thin layer chromatography) and methacryloyl chloride were purchased Sigma (St Louis, MO). Hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA) 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. All other chemicals were of reagent grade and were supplied by Merck AG (Darmstadt, Germany). 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.

### Synthesis of MAH

The MAH was selected as the metal-complexing ligand. Synthesis and characterization of MAH was described in our previous article.[29] The following experimental procedure was applied for the synthesis of MAH. 5.0 g of L-histidine methyesther and 0.2 g of hydroquinone were dissolved in 100 mL of dichloromethane solution. This solution was cooled down to 0°C. Totally, 12.7 g triethylamine was added to the solution. About 5.0 mL of methacryloyl chloride was poured slowly into this solution and then this solution was stirred magnetically at room temperature for 2 h. At the end of the chemical reaction period, hydroquinone and unreacted methacryloyl chloride were extracted with 10% NaOH solution. Aqueous phase was evaporated in a rotary evaporator. The residue (i.e., MAH) was crystallized in an ether-cyclohexane mixture and then dissolved in ethyl alcohol.

### Preparation of PHEMAH membranes

Production of PHEMAH membrane was performed using the Denizli et al.'s procedure.[30] Briefly, AIBN (5 mg) and *N*-methacryloyl-(L)-histidine methyl ester (MAH, 100 mg) were dissolved in HEMA monomer (2 mL). Then, 3 mL of 0.1*M* $SnCl_4$ was added into this mixture. Later, this polymerization mixture was degassed under vacuum for 5 min to eliminate soluble oxygen. 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 ethyl alcohol, and cut into circular pieces (0.5 cm in diameter, thickness: 350 μm) with a perforator. After washing with 200 mL of water, the membrane pieces were stored in a buffer containing 0.02% sodium azide ($NaN_3$) at 4°C until use.

### Characterization of membranes

Swelling ratio was determined in distilled water. The experiment was conducted as follows: initially dry membrane (diameter: 1 cm) were carefully weighed before being placed in a 50 mL vial containing distilled water. The vial was put into a water bath at 25°C for 2 h. The swollen membrane was taken out from the water periodically, wiped using a filter paper, and weighed. The water content of the membrane was calculated by using the following expression:

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

where $W_o$ and $W_s$ are the weights of membrane before and after swelling, respectively.

Microscopic observations and photographs of the gold coated PHEMAH membrane was performed by using a scanning electron microscope (SEM, Model: Raster Electronen Microscopy, Leitz-AMR-1000, Germany).

The thickness of the PHEMAH membrane was measured using a micrometer thickness gauge.

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 (Italy). The surface area of the membrane sample was measured with a surface area apparatus (BET method).

To evaluate the degree of MAH incorporation, the synthesized PHEMAH membranes were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932).

The membranes were characterized by the air under water contact angle measuring technique. This device consists of a traveling goniometer with 15× eyepieces a variable intensity light source and a micrometer-adjustable X-Y stage vertically mounted on an optical bench. The stage contains a Plexiglas container in which a Teflon plate is suspended.

The polymer sample is held on the underside of the Teflon plate by means of small Teflon clips. The container is then filled with triple distilled water and the plate with sample lowered into the container until the sample is completely immersed. A bubble of air with a volume of about 0.5 μL is then formed at the tip of the Hamilton microsyringe, below the surface, detached and allowed to rise to the polymer–water interface. The air bubbles were photographed at 25°C within 5 min to reach equilibrium after contact with the membranes. The equilibrium contact angle (qair) was calculated from the height (*h*) and the width (*b*) of the air bubble at the membrane surface. The mean value of five contact angles measured on bubbles at different positions is considered. The reproducibility of contact angles is ± 2%.

### Chelation of metal ions

Chelation of metal ions including $Zn^{2+}$, $Ni^{2+}$, $Co^{2+}$, and $Cu^{2+}$ from the single metal ions solutions was investigated in batch adsorption-equilibrium experiments. Aqueous metal ion solution (20 mL) was treated with the PHEMAH membranes for 2 h (equilibrium adsorption time). The flask was magnetically stirred at an agitation rate of 100 rpm at room temperature (25°C). Initial concentration of metal ion was 30 mg/L (458.7 μ*M* for $Zn^{2+}$, 509.3 μ*M* for $Co^{2+}$, 511.1 μ*M* for $Ni^{2+}$, and 472.4 μ*M* for $Cu^{2+}$). Medium pH was 5.0. Nitrate salts were used for preparing standard metal ion solutions. The concentration of the metal ions in the aqueous phase, after the desired treatment periods was measured by using a graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Germany). The instrument response was periodically checked with known metal solution standards. The experiments were performed in replicates of three and the samples were analyzed in replicates of three as well. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples to determine the margin of error.

### IgG adsorption from aqueous solution

IgG adsorption on the metal-chelated PHEMAH membranes were studied in batch wise. PHEMAH membranes loaded metal ions were incubated with 50 mL of IgG solution at 20°C for 2 h. The pH of the solution was 7.4. The initial concentration of IgG was 1.0 mg/mL. After the IgG adsorption, to remove the nonspecifically adsorbed IgG molecules, the PHEMAH membranes were washed with 0.1*M* borate buffer + 0.15*M* NaCl (pH 8.8) with 2*M* urea + 0.15*M* NaCl, and finally with 0.1*M* $NaHCO_3$ (pH 9.5) + 0.5*M* NaCl. The protein concentration in aqueous solutions was measured using Lowry method. The protein sample (500 μL) was diluted to 1.0 mL with phosphate buffered saline (PBS). Thereafter 1.0 mL of a freshly prepared Lowry reagent was added. After 30 min incubation at room temperature, 500 μL of freshly prepared Folin-Ciocalteu's reagent was added and the solution was mixed using a vortex. The blank solution was prepared analogously as the protein sample using 500 μL of a 3.0% SDS solution in PBS instead of the protein solution. After 30 min incubation at room temperature, the absorbance of the protein sample was measured at 730 nm against the blank solution using a Pharmacia LKB Spectrophotometer Novaspec II (Upsala, Sweden). A calibration curve was established using bovine serum albumin with known concentrations to relate the protein concentration in the solution with the absorbance of the sample. The IgG adsorption capacity of membranes was determined by measuring the remaining concentration of IgG in the adsorption medium.

### IgG adsorption from human plasma

Adsorption of IgG from human plasma on the metal-chelated PHEMAH membranes was studied in batch wise. Fresh human blood was used in all experiments. Fresh frozen plasma was obtained from University Hospital (Hacettepe, Ankara, Turkey). Blood samples were centrifuged at 3000 rpm for 30 min at room temperature to separate plasma. PHEMAH membranes loaded metal ions were incubated at 20°C for 230 min with 5 mL of human plasma. PBS (pH: 7.4, NaCl: 0.9%) was used for dilution of human plasma.

The amount of IgG adsorbed on the metal-ions loaded PHEMAH membranes was determined by a solid-phase-enzyme-linked immunosorbent assay method (ELISA). Human anti-IgG (Sigma, I-9384) diluted 1/1000 in 50 m*M* $NaHCO_3$, pH 9.6 was adsorbed to polyvinylchloride microtitre plates at 4°C for 12 h. The plates were washed with PBS containing 0.05% Tween 20 (wash buffer) and blocked with PBS containing 0.05% Tween 20, 1.5% bovine serum albumin (BSA and 0.1% sodium azide (blocking buffer). Samples (2.5 mL, neutralized with 0.5 mL of 1.0*M* trisodium citrate) or controls containing known amounts of IgG were added and incubated at 37°C for 1 h. Adsorbed IgG was detected with the anti human-IgG labeled with biotin (Sigma, B-3773) followed by peroxidase-conjugated streptavidin (Sigma) and *o*-phenylenediamine. The absorbance was measured at 492 nm.

SDS-PAGE analysis of the serum samples was performed on 10% separating mini gels (9 cm × 7.5 cm) for 120 min at 200 V. Stacking gels (6%) were stained

**TABLE I**
**Physical Properties of PHEMAH Membranes**

| | |
|---|---|
| Swelling Ratio (%) | 147.9 |
| Pore Radius (nm) | 1000–5000 |
| Surface area ($m^2/g$) | 14.6 |
| Membrane thickness (μm) | 350 |
| MAH content (μmol/g) | 168.5 |
| Contact Angle (°) | 41 |

with 0.25% (w/v). Coomassie Brillant R 250 in acetic acid–methanol–water mixture (1 : 5 : 5, v/v/v) and destained in ethanol–acetic acid–water mixture (1 : 4 : 6, v/v/v). The SDS-PAGE gels were scanned using a Shimadzu dual-wavelength flying spot scanning densitometer (Shimadzu, Tokyo, Japan).

### Elution and repeated use

Elution of IgG was studied in 250 m*M* ethylenediamine tetraacetic acid (EDTA). IgG adsorbed metal-chelated PHEMAH membranes were placed in elution medium and stirred continuously (at a stirring rate of 750 rpm) for 1 h at room temperature. The total volume of elution medium was 50 mL. The final IgG concentration in the elution medium was determined by ELISA. The elution ratio was calculated from the amount of IgG adsorbed on the metal-chelated PHEMAH membranes and the final IgG concentration in the elution medium.

To test the reusability of the metal-chelated PHEMAH membranes, IgG adsorption-elution procedure was repeated 10 times by using the same polymeric support. After each IgG adsorption-elution experiment, the metal ions were stripped with 250 m*M* EDTA at pH 4.9, and the metal ion chelation procedure was applied again. It should be also noted that, after elution of IgG, metal ion leakage from the metal-chelated PHEMAH membranes was also monitored continuously.

## RESULTS AND DISCUSSION

### Properties of PHEMAH membranes

Several physicochemical properties of PHEMAH membranes are presented in Table I. PHEMA belongs to a class of polymers known as hydrogels, which swell in contact with water. Hydrophilic PHEMAH membranes prepared in this study swell in water, but do not dissolve. The scanning electron microscope (SEM) photographs show the surface structure and the cross section of the PHEMAH membranes (Fig. 1). As seen from the surface and cross-sectional surface photographs, the membranes have large pores. The membrane surface seems very rough and heterogeneous.

### IgG adsorption from aqueous solutions

#### Effect of metal type

Different metal affinity interaction mechanisms with proteins have been proposed,[31] but still the macromolecular recognition of proteins with chelated metal ions obviously remains unclear. In one proposed mechanism, the formation of a coordination complex between protein and chelated metal ion is considered to be the major binding mode. However, more than one type of interaction mechanism is operational.[32] The major functional groups on protein contributing toward the interaction with chelated 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, the exposed histidine residue is the dominant binding site in protein adsorption with a chelated metal ion.[33] Factors influencing the interactions include the number of electron-donating groups on the protein surface, medium pH, concentration of protein, type of metal

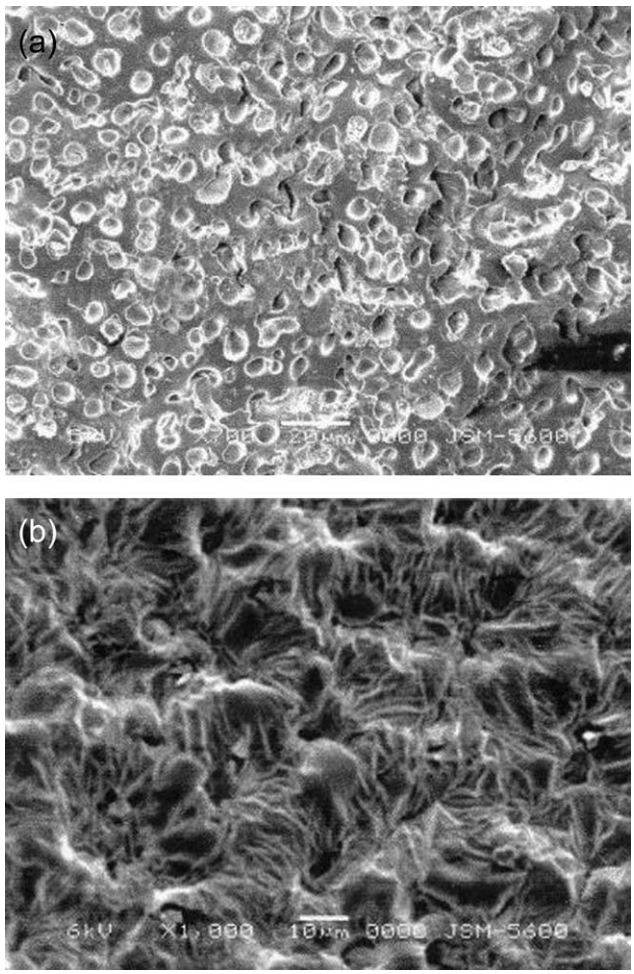
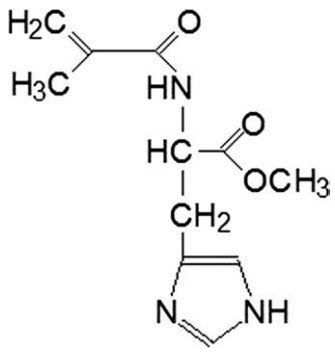



**Figure 1** The SEM images of PHEMAH membranes (a) Surface; (b) Cross-sectional area.




**Scheme 1** Molecular structure of MAH.

ions, ligand density, type, and size of chelating ligand.

Imidazole nitrogen donor atom in MAH is the most common binding site for metal ions. Molecular structure of MAH was given in Scheme 1. Figure 2 shows the effects of metal ion incorporation on IgG adsorption from aqueous solutions. IgG adsorption capacity on PHEMA membranes was 0.25 mg/g. It should be noted that a negligible amount of protein adsorbed nonselectively on the membranes is one of the most important requisites. Hydrated water layer on the polymer surface will reduce the hydrophobic interactions between the protein and the polymer. The maximum IgG binding capacity of PHEMAH membranes was found to be 12.4 mg/g. IgG adsorption capacities on the metal-chelated PHEMAH membranes were found to be 28.5 mg/g for $Co^{2+}$, 32.3 mg/g for $Zn^{2+}$, 39.0 mg/g for $Ni^{2+}$, and 44.4 mg/g for $Cu^{2+}$ ions under the same conditions. We have shown that metal ion incorporation significantly increased the IgG adsorption capacity of the

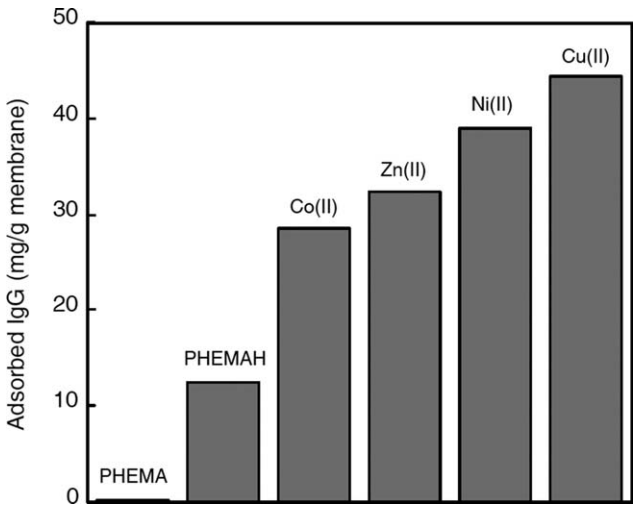



**Figure 2** Effect of metal ion type on IgG adsorption on the PHEMAH membranes; MAH loading: 168.5 μmol/g; Metal loading values: 78.9 μmol/g for $Zn^{2+}$, 99.5 μmol/g for $Co^{2+}$, 115.6 μmol/g for $Ni^{2+}$ and 122.5 μmol/g for $Cu^{2+}$; IgG concentration: 1.5 mg/mL; T: 25°C.

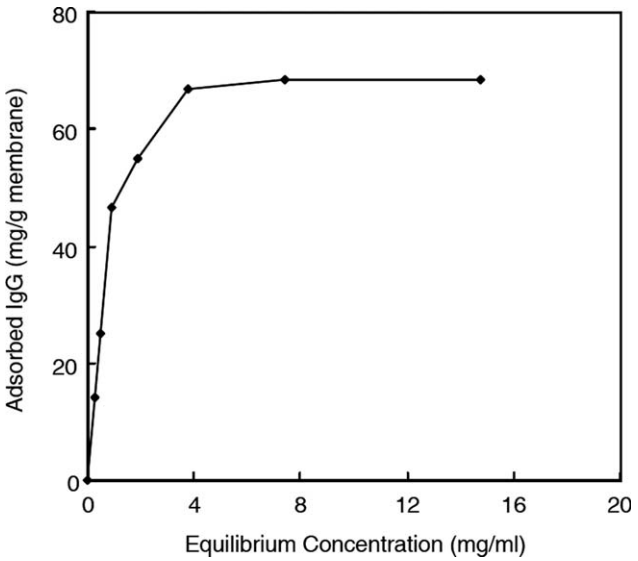



**Figure 3** IgG adsorption from human plasma on PHEMAH membranes; MAH loading: 168.5 μmol/g; T: 25°C.

membranes. Transition metal ions have a high affinity to the peptide sequences His-Gly-His, His-Tyr-NH, and His-Trp.[34] This significant IgG adsorption onto the metal-chelated PHEMAH membranes could be due to its greater number of histidine residues, which are interacting with the proteins. IgG adsorption capacity was showed towards the metal ion with the effects in the order: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$.

### IgG adsorption from human plasma

Figure 3 and 4 shows the adsorption data on the PHEMA and the metal-chelated PHEMAH membranes. IgG adsorption onto the PHEMAH membranes through MAH molecules was significant (up to 68.4 mg/g), and increased with increasing the equilibrium concentration of IgG in the human plasma. MAH in the polymer structure proved useful also for the capture of IgG directly from healthy human plasma. The IgG molecules were found to be adsorbing on MAH via their Fab part.[35] The adsorbed amount of IgG onto the PHEMAH membranes via MAH reached almost a plateau value around 4.0 mg/mL, due to the saturation of active binding sites (Fig. 3). There was a further increase in IgG adsorption capacity from human plasma with the metal-chelated PHEMAH membranes. The maximum IgG adsorption capacity of metal-chelated PHEMAH membranes were 118.0 mg/g for $Cu^{2+}$, 89.2 mg/g for $Ni^{2+}$, 76.5 mg/g for $Zn^{2+}$, and 72.5 mg/g for $Co^{2+}$ ions under the same conditions (Fig. 4). It was shown that metal ion chelation significantly increased the IgG adsorption capacity of the PHEMAH membranes. This could be due to the

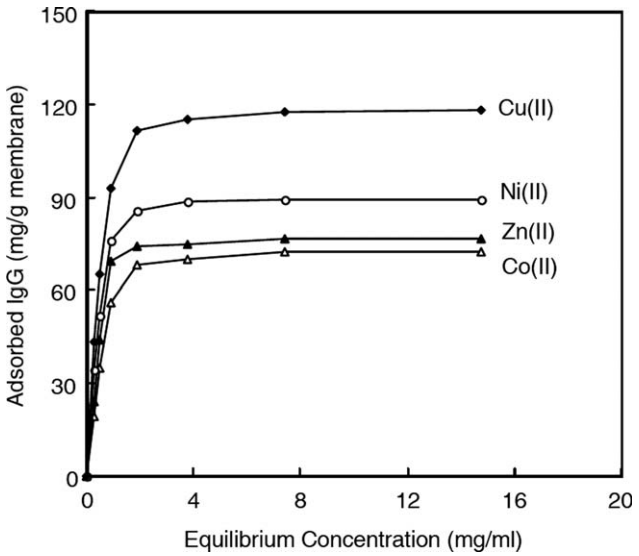



**Figure 4** IgG adsorption from human plasma on metal-chelated PHEMAH membranes; MAH loading: 168.5 μmol/g; Metal loading values: 78.9 μmol/g for $Zn^{2+}$, 99.5 μmol/g for $Co^{2+}$, 115.6 μmol/g for $Ni^{2+}$, and 122.5 μmol/g for $Cu^{2+}$; T: 25°C.

greater number of surface accessible histidine residues present in IgG, which interacted with the chelated metal ions.[36] Hale and Beidler described a histidine rich region in the third constant domain of heavy chain (CH3) of IgG.[37] Further molecular modeling studies performed by Todorova-Balvay et al. revealed the accessibility of histidine residues located in the Fc region of IgG.[38] They showed the presence of histidine cluster His 433-X-His 435 in the Fc portion of IgG and both these histidine residues were accessible for interaction with metal ions. The recognition range of metal ions for surface histidines followed the order: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$. This affinity trend agrees well with reported tendencies of chelated metal ions for various surface histidine residue distributions. Each of the four metal ions can coordinate up to six electron donors, and their affinity towards imidazole follows the order $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$.[39,40] A protein needs at least two histidines to be adsorbed on a $Ni^{2+}$, $Zn^{2+}$, and $Co^{2+}$ chelated adsorbents, whereas $Cu^{2+}$-chelated adsorbents can recognize any histidine distribution, even single histidine on a protein surface.

The purity of IgG eluted from the metal-chelated PHEMAH membranes was determined by SDS-PAGE as given in experimental section. The purified IgG was compared with standard protein. As clearly shown in Figure 5, IgG in serum (Lane 4) was almost disappeared in Lane 3 after adsorption onto the PHEMAH membranes. Furthermore, the presence of only single band of IgG at Lane 3 confirms the success of purification procedure and also indicates the purity of IgG after elution of PHEMAH membrane. The purity of IgG obtained was 94.1%. The recovery of IgG from human plasma was 84.5%.

There have been many attempts to develop new materials with high stability an affinity towards immunoglobulins. A comparison of maximum adsorption capacity of metal-chelated PHEMAH membranes with those of some other affinity materials reported in literature is given in Table II. The adsorption capacity of the metal chelated PHEMAH membranes is comparable even higher than the capacity of other materials including commercially available carriers.

### Elution of IgG

In addition to increased capacity, the other major area of concern for affinity carriers is that the

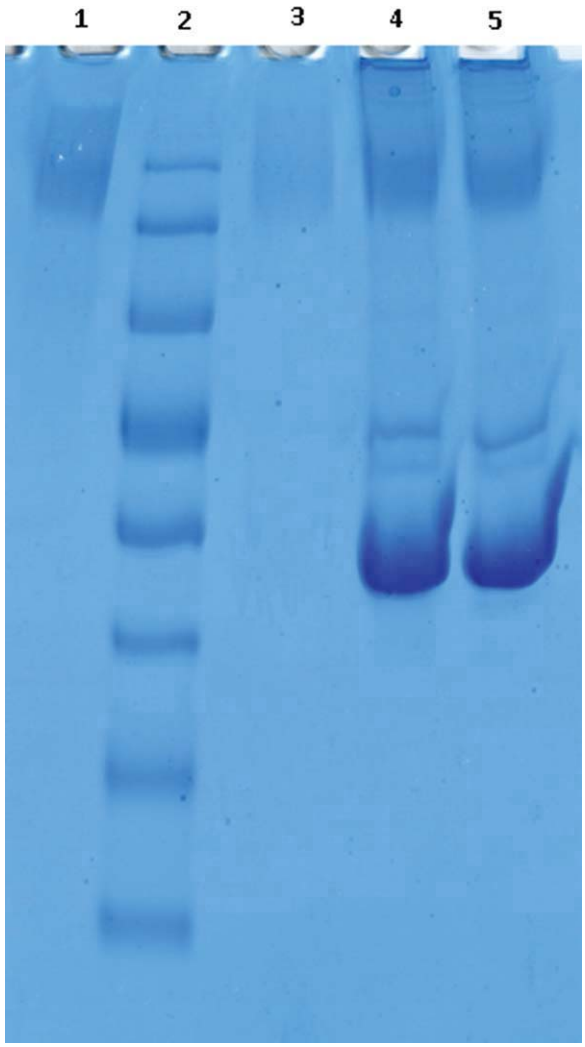



**Figure 5** SDS-PAGE of serum fractions. Lane 1, IgG (150 kDa); Lane 2, biomarker (Fermentas); Lane 3, eluted sample. Lane 4, 1/10 diluted serum; Lane 5, 1/10 diluted serum after adsorption; Equal amounts of samples were applied to each lane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**TABLE II**
**Comparison of the Adsorption Capacities for IgG of Various Carriers**

| Adsorbent | Ligand | Ligand Loading | $Q_{max}$ (mg/g) | Reference |
|---|---|---|---|---|
| PHEMA beads | Mannosylerythritol | 4 mg/g | 12.6 | 1 |
| PHEMA cryogel | Protein A | 56 mg/g | 98.7 | 6 |
| Eupergit, Affigel beads | Protein A | 4.1 mg/mL | 20.1 | 9 |
| PHEMA beads | L-Histidine | – | 44.8 | 27 |
| CIM monolith | IDA/$Cu^{2+}$ | – | 15.4 | 36 |
| PHEMA/L-Histidine | $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Co^{2+}$ | 1.2 mmol/g | 79.6 | 40 |
| PHEMA beads | Protein A | 2.7 mg/g | 24.0 | 41 |
| Agarose | w-Aminodecyl | – | 19.8 | 42 |
| PHEMA beads | Concanavalin A | 56.8 mg/g | 69.4 | 43 |
| Polymethylmethacrylate | $Cu^{2+}$ | 39.5 μmol/g | 54.3 | 44 |
| Polyhydroxypropylmethacrylate | Reactive Green HE4BD | 44.3 μmol/g | 86.6 | 45 |
| StreamlineTM | Reactive Green 5 | 17.4 μmol/ml | 49.0 | 46 |
| Bentonite particles | L-Histidine | 541.3 μmol/g | 89.6 | 47 |
| Poly(HEMA-EGDMA) beads | IMEO | 36.6 mg/g | 78.9 | 48 |
| PHEMA nanoparticles | IMEO | 64.5 mg/g | 843 | 49 |
| Poly(DVB-VAc) | 2-Mercaptonicotinic acid | 105 μmol/g | 78 | 50 |
| P(HEMA-MAPA) nanoparticles | Pheylalanine | 420 μmol/g | 780 | 51 |
| Poly(St-VAc) | Mercaptopyrimidine | 110 μmol/g | 19 | 52 |
| Poly(AAm-AGE) cryogel | Concanavalin A | 12.8 mg/g | 25.6 | 53 |
| PHEMA monolith | $Cu^{2+}$ | 16.3 μmol/g | 104.2 | 54 |
| PHEMA monolith | MAH | 47.8 μmol/g | 96.5 | 55 |
| PHEMA membrane | Protein A | 4.7 mg/g | 9.8 | 56 |
| Poly(EGDMA) beads | Histidine | 70 μmol/g | 320 | 57 |
| Poly(GMA) beads | IDA/$Cu^{2+}$ | 628 μmol/g | 171.2 | 58 |
| PHEMA/Histidine membrane | $Cu^{2+}$, $Ni^{2+}$, $Zn^{2+}$, $Co^{2+}$ | 78.9–122 μmol/g | 118 | In this study |

stability to regeneration.[59] The adsorbed IgG was eluted from the metal-chelated PHEMAH membranes by incubation in 250 m*M* EDTA solution for 1 h. It should be noted that human plasma was used for repeated IgG adsorption cycles. Maximum elution ratio was 96%. It must be pointed out that in metal chelated affinity systems, binding of proteins with chelated ions are completely reversible. Note that there is no MAH release in this case which shows that MAH is polymerized with HEMA monomer.

To show the reusability of the metal-chelated PHEMAH membranes, the adsorption–elution cycle was repeated ten times using the same polymeric membranes. There was no remarkable reduce in the adsorption capacity of the adsorbents (Fig. 6). The IgG adsorption capacity decreased only 1.8% after 10 cycles. This increased lifetime has a direct impact on the process economics of the purification process. With the elution data given above we concluded that EDTA is a suitable elution agent, and allows repeated use of the metal-chelate affinity membranes used in this study.

## CONCLUSIONS

Immunoglobulins are widely used for both *in vitro* diagnostic purposes and inducing passive immunity.[60–63] The commercial relevance of immunoglobulins has stimulated the development of cost and time effective purification techniques. Protein A columns cannot be used for large scale purification, since they are very expensive. Pseudospecific adsorbents may hold certain advantages for industrial affinity separations since the ligands are not likely to cause an immune response in case of leakage into the product.[64–68] An expensive step in the

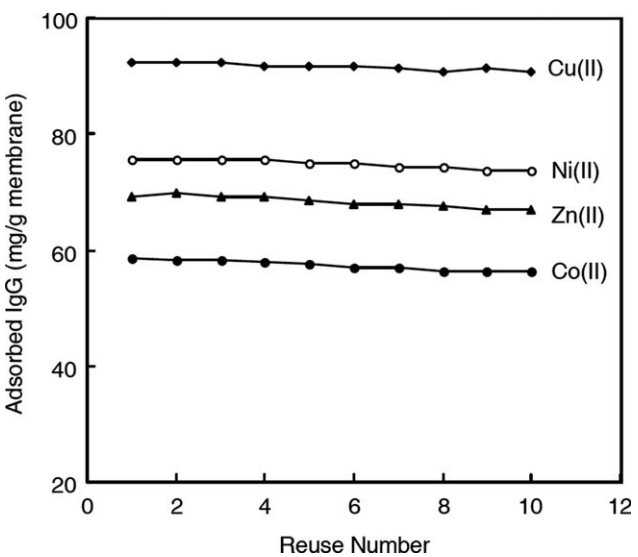



**Figure 6** Repeated use of metal-chelated PHEMAH membranes; MAH loading: 168.5 μmol/g; Metal loading values: 78.9 μmol/g for $Zn^{2+}$, 99.5 μmol/g for $Co^{2+}$, 115.6 μmol/g for $Ni^{2+}$, and 122.5 μmol/g for $Cu^{2+}$; IgG concentration: 1.5 mg/mL; T: 25°C.

preparation process of metal-chelating adsorbent is coupling of a chelating ligand to the adsorption matrix. In this article, MAH is used as the metal-chelating ligand, and there is no need to activate the matrix for the chelating-ligand immobilization. Another major problem is slow release of chelators from the matrix which caused a decrease in adsorption capacity. Metal-chelating ligand immobilization step is also eliminated in this approach. MAH is polymerized with HEMA. PHEMAH membranes resulted a IgG adsorption of 12.4 mg/g from aqueous solutions. A remarkable increase in the IgG adsorption capacities are achieved from human plasma (up to 68.4 mg/g). A further increase is achieved from human plasma with metal chelation (up to 118 mg/g). The recognition range of metal ions for surface histidine from human plasma followed the order: $Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+}$. This affinity trend agrees well with reported tendencies of chelated metal ions for various surface histidine residue distributions. High elution ratios (more than 96% of the adsorbed IgG) were achieved by using 250 m*M* EDTA. It is possible to reuse these metal-chelated affinity membranes without remarkable reduce in the adsorption capacities.

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