Pseudospecific Magnetic Affinity Beads for Immunoglobulin-G Depletion from Human Serum

Sinan Akgöl,[1] Serpil Özkara,[2] Lokman Uzun,[2] Fatma Yılmaz,[2] Adil Denizli[2]

[1]*Biochemistry Division, Department of Chemistry, Adnan Menderes University, Aydın, Turkey*
[2]*Biochemistry Division, Department of Chemistry, Hacettepe University, Ankara, Turkey*

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ABSTRACT: Magnetic poly(ethylene glycol dimethacrylate-*N*-methacryloyl-(L)-histidine methyl ester) [m-poly(EGDMA-MAH)] beads were prepared by suspension polymerization for the affinity depletion of immunoglobulin-G (IgG) from human serum in a batch system. Elemental analysis of the magnetic beads for nitrogen was estimated as 70 μmol MAH/g polymer. IgG adsorption onto the m-poly(EGDMA) was negligible. Higher adsorption value (up to 46.8 mg/g) was obtained in which the m-poly(EGDMA-MAH) beads were used. IgG adsorption capacity of the magnetic beads increased with an increase in the concentration of IgG. The maximum IgG adsorption was observed at pH 6.5 for MOPS buffer. IgG molecules could be repeatedly adsorbed and eluted with these adsorbents, without noticeable loss in their IgG adsorption capacity. Adsorption capacity decreased for both increasing salt concentration and temperature. In this study, we show that m-poly(EGDMA-MAH) beads (wherein IgG molecules bind directly with the matrix) can be used directly for affinity depletion without further modification. Higher adsorption value was obtained from human serum (up to 85.7 mg/g). The elution results demonstrated that the adsorption of IgG to the adsorbent was reversible. The depletion efficiencies for IgG were above 85% for all studied concentrations. Eluted portion was analyzed for testing the IgG removal efficiency by two dimensional gel electrophoresis. Eluted proteins include mainly IgG, and a small number of nonalbumin proteins such as apolipoprotein A1, serotransferrin, haptoglobulin, and α1-antitrypsin. IgA was not identified in eluted fraction. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2405–2412, 2007

Key words: protein depletion; pseudospecific adsorbents; affinity chromatography; magnetic beads; proteomics

INTRODUCTION

Serum plays a central role in clinical diagnosis. Serum is thought to contain tens of thousands of proteins along with their cleaved or modified forms. These proteins are a reflection of ongoing physiological or pathological events.[1] Serum proteins may often serve as indicators of disease and is a rich source for biomarker discovery. However, the large dynamic range of proteins in serum makes the analysis very challenging, because high abundant proteins (tens to ~ 2 mg/mL) including albumin, immunoglobulins (IgG and IgA), antitrypsin, haptoglobin, and transferrin tend to mask of those of lower abundance.[2] Collectively, albumin and immunoglobulin constitute over 80% of the human serum proteome.[3] Therefore, removal of these proteins represents a fundamental improvement toward characterization of the serum proteome.[4]

Correspondence to: A. Denizli (denizli@hacettepe.edu.tr).
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Currently, there are several depletion technologies to remove the higher abundant proteins from serum, including ultracentrifugal filtration, dye affinity, and immunoaffinity depletion.[5–10] The removal of IgG is commonly achieved by protein A/G affinity adsorbents, which binds to the Fc region of the IgG,[11,12] but specific antibodies can also be used. Protein A/G and monoclonal antibodies to IgG are available from commercial suppliers. However, IgG is present in serum at concentrations in the range of 8–16 mg/mL, and large quantities of antibody and/or protein A/G are required for its quantitative removal. The high specificity of the bioligands provides excellent selectivity. However, in spite of their high selectivities, protein A/G or antibody carrying adsorbents also have some drawbacks that are worth considering: (i) the cost of these materials tends to be very high; (ii) These bioligands are difficult to immobilize in the proper orientation. In addition, the depletion of IgG in human serum is employed for the treatment of immune disorders, including systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, alloimmunization, and cancer.[13–19]

Recently, magnetic beads are currently enjoying a fairly ample range of applications in many fields including biotechnology, biochemistry, colloid scien-

ces, and medicine among others.[20–25] The magnetic character implies that they respond to a magnet, making sampling and collection easier and faster, but their magnetization disappears once the magnetic field is removed. Magnetic beads promises to solve many of the problems associated with chromatographic separations in packed bed and in conventional fluidized bed systems.[26] Magnetic separation is relatively rapid and easy, requiring a simple apparatus composed of centrifugal separation.[27]

In this study, we report the development of a magnetic poly(ethylene glycol dimethacrylate-*N*-methacryloyl-(L)-histidinemethylester) [m-poly(EGDMA-MAH)] adsorbent containing a paramagnetic component. We have assessed the suitability of the m-poly(EGDMA-MAH) beads for removal of IgG. Elution of IgG and regeneration of the magnetic beads were also tested.

MATERIALS AND METHODS

Materials

Purified human-immunoglobulin G (IgG, lyophilized) was purchased from Sigma Chemical Company (St Louis, MO). The purity of this material as specified by the supplier was greater than 95% based on nonreduced sodium dodecyl sulphate–polyacrylamide gel electrophoresis. L-Histidine methylester, methacryloyl chloride, morpholinopropanesulfonic acid (MOPS), hydroxyethylpiperazineethanesulfonic acid (HEPES), and morpholinoethanesulfonic acid (MES) were supplied by Sigma. 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. Magnetite particles (Fe_3O_4, diameter < 5 μm) were obtained from Aldrich. Benzoylperoxide (BPO) was obtained from Fluka. Poly(vinyl alcohol) (PVAL; MW: 100,000, 98% hydrolyzed) was supplied from Aldrich. All other chemicals were purchased from available commercial sources and used as obtained. 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 pseudospecific affinity ligand. Synthesis and characterization of MAH was described in our previous article.[28] The following experimental procedure was applied for the synthesis of MAH. L-Histidine methylester (5.0 g) and 0.2 g of hydroquinone were dissolved in 100 mL of dichloromethane solution. This solution was cooled down to 0°C. Triethylamine (12.7 g) was added to the solution. Methacryloyl chloride (5.0 mL) 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. The ^{1}H NMR spectrum of MAH 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 (δ) downfield relative to $CHCl_3$. ^{1}H NMR spectroscopy was used to determine the structure of MAH. Characteristic peaks are as follows: ^{1}H NMR ($CDCl_3$): δ = 1.99 (t; 3H, J = 7.08 Hz, CH_3), 1.42 (m; 2H, CH_2), 3.56 (t; 3H, O—CH_3) 4.82–4.87 (m; 1H, methin), 5.26 (s; 1H, vinyl H), 5.58 (s; 1H, vinyl H); 6.86 (δ; 1H, J = 7.4 Hz, NH), 7.82 (δ; 1H, J = 8.4 Hz, NH), 6.86–7.52 (m; 5H, aromatic).

Preparation of magnetic poly(EGDMA-MAH) beads

Synthesis and characterization of m-poly(EGDMA-MAH) beads was described in our previous article.[29] A typical procedure may be summarized as follows: The stabilizer, PVAL, was dissolved in 50 mL deionized water for the preparation of the continuous phase. The dispersion phase was prepared by mixing EGDMA (8.0 mL), MAH (1.0 g), toluene (12.0 mL), and magnetite particles (1.0 g) in a test tube. The initiator, BPO (100 mg), was dissolved in this homogeneous solution. The dispersion phase was added to the continuous medium in a glass-sealed polymerization reactor (100 mL) placed in a water bath equipped with a temperature-control system. The polymerization reactor was heated to 65°C within about 30 min by stirring the polymerization medium at 600 rpm. The polymerizaton was conducted at 65°C for 4 h and at 90°C for 2 h. After completion of polymerization, the reactor content was cooled to room temperature. m-Poly(EGDMA) beads were obtained by the same procedure without adding MAH comonomer into the polymerization medium. A washing procedure was applied after polymerization to remove the diluent and any possible unreacted monomers and other ingredients from the beads. The magnetic beads were filtered and resuspended in ethyl alcohol. The suspension was stirred for about 1 h at room temperature and the beads were separated by filtration. The beads were washed twice with ethyl alcohol and then four times with deionized water using the same procedure. When not in use, the magnetic beads were kept under re-

frigeration in 0.02% sodium azide solution for preventing of microbial contamination.

Batch procedure

Protein adsorption studies were carried out in a batch system. Magnetic beads suspended in pure water were degassed under reduced pressure (by using water suction pump). The beads were incubated with 10 mL of IgG solution for 2 h, in flasks agitated magnetically at 150 rpm. Effects of IgG concentration, medium pH, buffer type, temperature, and ionic strength on the adsorption capacity were studied. To observe the influence of buffer to the adsorption capacity, the adsorption studies were carried out using 25 m*M* MES, MOPS, HEPES, Tris-HCl, and phosphate buffers (contains 0.15*M* NaCl) within their respective buffering ranges. To observe the effects of the initial concentration of IgG on adsorption, it was changed between 0.1 and 2.0 mg/mL. IgG concentration determined by measuring the absorbance at 280 nm, with a molar absorptivity of 14.0 for a 1% solution of IgG. The amount of adsorbed IgG per dry magnetic beads was calculated by using the concentrations of the IgG in the initial solution and in the equilibrium.

Elution and repeated use

Elution of IgG was studied with 1*M* NaCl at pH 4.0 in acetate buffer. The IgG-adsorbed magnetic beads were placed in this elution medium and stirred continuously at 600 rpm at room temperature for 1 h. The final IgG concentration in the elution medium was determined spectrophotometrically. The elution ratio was calculated from the amount of IgG adsorbed on the magnetic beads and the final IgG concentration in the elution medium. In order to test the reusability of the magnetic beads, adsorption–elution was repeated 10 times by using the same polymeric adsorbent. In order to regenerate and sterilize, the magnetic beads were washed with 50 m*M* NaOH solution after the elution.

IgG depletion from human serum

IgG depletion from human serum with m-poly-(EGDMA-MAH) beads was studied batchwise. The blood is collected from thoroughly controlled voluntary blood donors. Each unit separately controlled and found negative for hepatit B-specific antigen and HIV I, II, and hepatitis C antibodies. No preservatives are added to the samples. Blood samples were centrifuged at 500 $\times$ *g* for 3 min at room temperature to separate the serum. The serum samples were filtered using 0.45-μm cellulose acetate microspin filters (Alltech, Deerfield IL). The concentration of IgG in crude original serum was determined to 14.3 mg/mL by nephelometric assay. Total protein content of crude and depleted serum samples was determined using the DC Protein Assay (Bio-Rad) according to the manufacturers instructions, with IgG as standard (Pierce, Rockford, IL). Total protein concentration in crude serum was 62 mg/mL. In order to deplete human serum albumin (HSA), the freshly separated human serum (100 mL) was loaded onto a anti-HSA antibody-sepharose column (10 cm $\times$ 1 cm inside diameter) equipped with a water jacket for temperature control. Equilibration of anti-HSA antibody-sepharose column (Sigma) was performed by passing four column volumes of sodium acetate buffer (pH: 5.2) before injection of the serum. When serum passes through the column, the HSA molecules were adsorbed on the anti-HSA antibody-sepharose adsorbent. The albumin-free serum which passed from the column consists mainly of IgG and other serum proteins. After that the serum is ready for pseudospecific affinity depletion of IgG.

HSA concentration was determined using Ciba Corning Albumin Reagent (Catalog Ref. No: 229241) based on bromocresol green dye method. The concentration of HSA in crude serum was determined to be 40.2 mg/mL. The concentration of the remaning HSA in serum sample was very low. The percentage of albumin depletion was greater than 99.4%. Then, 25 mL of the albumin-free serum was incubated with 250 mg of magnetic-beads preequilibrated with acetate buffer (pH 5.0) for 2 h. The experiment was conducted at 20°C. The amount of IgG adsorbed by pseudospecific mag-beads was determined by measuring the initial and final concentration of IgG in serum. Analysis of IgG was performed by a nephelometer assay (Beckman Array 360, USA). Phosphate buffered saline (pH: 7.4, NaCl: 0.9%) was used for dilution of human serum. In order to test the binding performance, two-dimensional gel electrophoresis (2DE) was carried out as described in detail previously.[30]

RESULTS AND DISCUSSION

Figure 1 shows the scanning electron micrograph of the prepared m-poly(EGDMA-MAH) beads, illustrating that the beads are spherical in size with a diameter of 50–100 μm and porous structure. m-Poly-(EGDMA-MAH) beads are hydrophilic networks capable of imbibing large amounts of water, yet remain insoluble and preserve their three-dimensional shape. The molecular formula of m-poly-(EGDMA-MAH) beads is given in Scheme 1. The equilibrium swelling ratio of the m-poly(EGDMA-MAH) beads is 40%. MAH groups in the polymer structure were evaluated by elemental analysis. Incorporation of MAH into the polymer structure

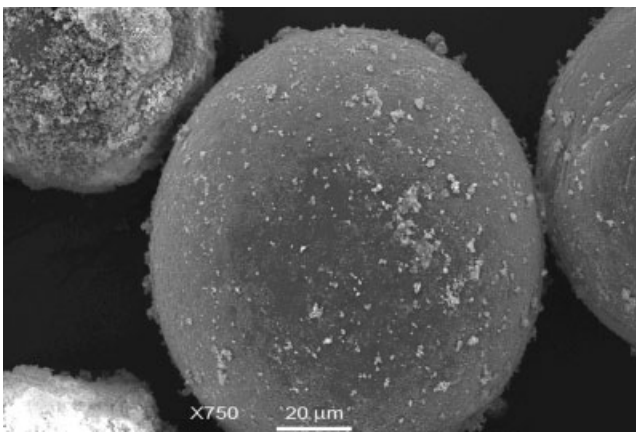


Figure 1 SEM photograph of m-poly(EGDMA-MAH) beads.

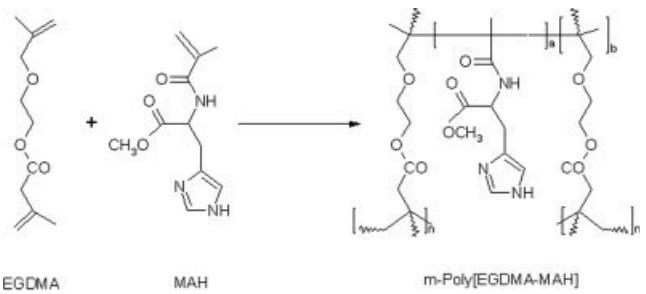
has minimized the ligand leaching problem and the ability to reuse the affinity beads helps to keep the cost reasonable for research purposes. The physicochemical properties of m-poly(EGDMA-MAH) beads are presented in Table I.

The choice of magnetic beads for affinity depletion is a critical factor for this methodology. In addition to the properties listed under Materials and Methods, the m-poly(EGDMA-MAH) beads that were prepared have surface-to-weight ratio of 80 m^2/g, potentially making for much higher binding capacities than any flat surfaces.

The hydrated density of the m-poly(EGDMA-MAH) beads measured at 25°C was 1.08 g/mL. By the same procedure, the density of magnetic Fe_3O_4 particles was found to be 1.97 g/mL at 25°C. The density of nonmagnetic poly(EGDMA-MAH) beads measured at 25°C was 1.03 g/mL. The poly(EGDMA-MAH) volume fraction in the magnetic beads can be calculated from the following equation derived from the mass balance:

$$\varphi = (\rho_C - \rho_M)/(\rho_C - \rho_A) \quad (1)$$

where, ρ_A, ρ_C, and ρ_M are the densities of poly(EGDMA-MAH) beads, magnetite Fe_3O_4 particles, and the m-poly(EGDMA-MAH) beads, respectively.



Scheme 1 The molecular formula of m-poly(EGDMA-MAH) beads.

TABLE I
Some Properties of the m-Poly(EGDMA-MAH) Beads

Particle diameter	50–100 µm
Average pore size	870 nm
Specific surface area	80 m^2/g
Swelling ratio	40%
MAH content	70 µmol/g
Wet density	1.08 g/mL
Fe_3O_4 incorporation	5.4%
Resonance of magnetic field	2400 Gauss
g factor	2.56

Thus with the density data mentioned earlier, the poly(EGDMA-MAH) gel volume fraction in the magnetic beads was estimated to be 0.946. In other words, the volume fraction magnetite of particles in the magnetic poly(EGDMA-MAH) beads was 0.054.

Effect of buffer type

Figure 2 shows IgG adsorption capacity in different buffer systems at different pHs. Buffer ranges are 5.0–6.5 for MES, 5.0–8.0 for phosphate, 7.0–8.0 for HEPES, 7.0–8.5 for Tris-HCl, and 6.0–8.0 for MOPS. In MOPS buffer, adsorption capacity is higher than other buffers. Maximum adsorption capacities are observed at pH 6.5 for MOPS (38.8 mg/g), at pH 7.4 for HEPES (27.2 mg/g), at pH 7.4 for Tris-HCl (19.6 mg/g), at pH 6.0 for phosphate (21.8 mg/g), and at pH 5.5 for MES (28 mg/g). Below and over the maximum adsorption pHs, adsorption capacities decreased significantly. It was shown pseudospecific affinity chromatography with m-poly(EGDMA-MAH) beads under zwitterionic buffers systems, like MOPS, MES, and HEPES, yielded

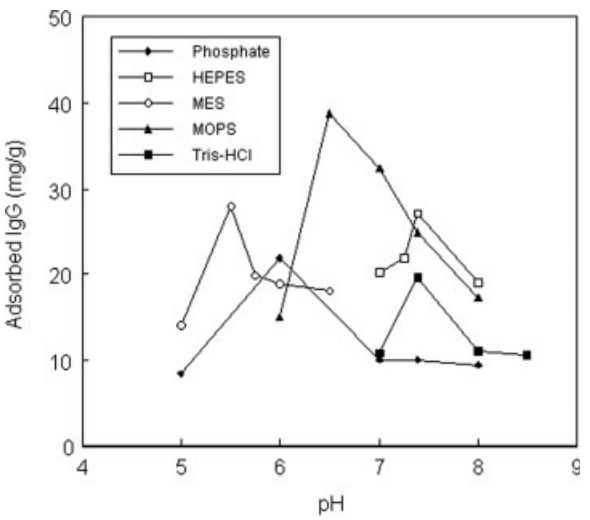


Figure 2 Effect of buffer type on IgG adsorption. IgG concentration: 0.5 mg/mL; MAH content: 70 µmol/g; *T*: 25°C.

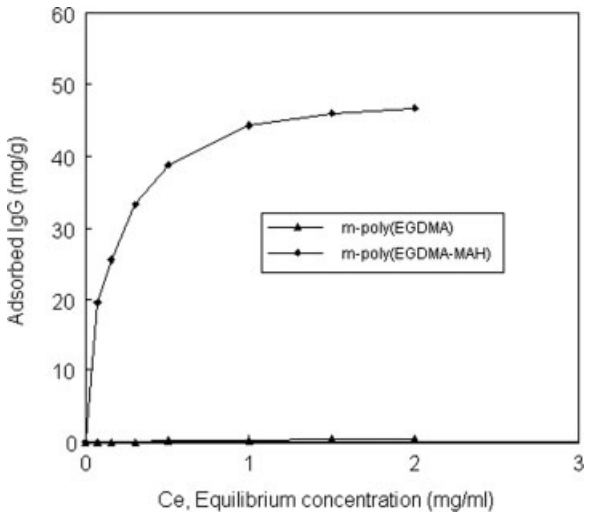


Figure 3 Adsorption equilibrium data in 25 m*M* MOPS buffer at pH 6.5; MAH content: 70 μmol/g; *T*: 25°C.

much higher adsorption capacities in comparison with other buffers like Tris-HCl and phosphate buffers. A similar result was obtained by Coffinier and Vijayalakshmi.[31] Increase in conformational size and the lateral electrostatic repulsions between adjacent adsorbed IgG molecules may also cause a decrease in adsorption efficiency below and over the maximum adsorption pH values. Also, the change of affinity interaction at high and low pHs should have a great influence. pK_a values for MOPS, HEPES, and MES are pH 6.5, 7.0 and 5.4, respectively. From the structure of the buffer ions used, it is obvious that Tris-HCl and phosphate carries one or more charge of the same sign—only (positive) or only (negative)—whereas the zwitter ionic buffers MES, MOPS, and HEPES carry two charges of opposite sign below their pK_a. It can clearly be observed that the maximum adsorption was obtained with MOPS and HEPES. A possible explanation of this behavior could be that MES, Tris-HCl, and phosphate buffer ions and the respective counter ions interact with the IgG molecules via charge–charge interactions and mask the binding site for histidine, but MOPS and HEPES in their zwitterionic form do not.

Effect of IgG concentration

Figure 3 shows the effect of IgG concentration on adsorption. As presented in this figure, with increasing IgG concentration, the amount of IgG adsorbed per unit mass increases below about 0.5 mg/mL, and then increases less rapidly. It reached saturation when the protein concentration is greater than 1.0 mg/mL. The steep slope of the initial part of the adsorption isotherm represents a high affinity between IgG and incorporated MAH groups. Negligible amount of IgG molecules was adsorbed on the hydrophilic m-poly(EGDMA) beads, which was about 0.4 mg/g. MAH incorporation significantly increased the IgG adsorption capacity of the beads up to 46.8 mg/g in MOPS buffer. It is clear that this increase is due to specific interaction between MAH groups and IgG molecules. The imidazole ring was known to have a mixed mode interaction mechanism by hydrophobic interaction, van der Waals forces, electrostatic interactions, and hydrogen bonding.[32] It should be also noted that the adsorption onto m-poly(EGDMA) beads were negligible for all buffers used.

During the experiments, adsorption isotherm was used to evaluate adsorption properties. The Langmuir adsorption isotherm is expressed by eq. (2). The corresponding transformations of the equilibrium data for IgG gave rise to a linear plot, indicating that Langmuir model could be applied in these systems and described by the equation:

$$q^* = q_{\max} C_{\text{eq}} / (K_d + C_{\text{eq}}) \tag{2}$$

where q^* is the amount of adsorbed IgG in the adsorbent, C_{eq} is the equilibrium IgG concentration in solution, K_d represents the dissociation constant, and $q_{\max}$ is the maximum adsorption capacity. This equation can be linearized so that

$$C_{\text{eq}}/q = K_d/q_{\max} + C_{\text{eq}}/q_{\max} \tag{3}$$

The plot of C_{eq} versus C_{eq}/q is employed to generate the intercept of $K_d/q_{\max}$ and the slope of $1/q_{\max}$.

The parameters, maximum adsorption capacity ($q_{\max}$) and K_d, were determined by nonlinear regression with commercially available software. It must be noted that the standard deviation of the values determined by regression analysis is comparatively low. The $q_{\max}$ data for the adsorption of IgG was obtained as 50.3 mg/g and K_d is found as 0.138 mg/mL from the experimental data (Fig. 4). The correlation coefficient (R^2) was 1.000 at pH 6.5 (for MOPS buffer). The Langmuir adsorption model can be applied in this affinity-adsorbent system.

Effect of ionic strength

Figure 5 shows the effect of ionic strength on the adsorption capacity. As seen here, adsorption capacity decreased with the increasing salt concentration. The adsorption of IgG decreases by about 90%, as the NaCl concentration changes from 0.01 *M* to 0.1*M*. There were two possible explanations to this phenomenon: (i) buffer ions and respective counter ions interact with the protein molecules via charge–charge interactions and mask the binding sites for histidine in MOPS buffer, (ii) decrease in the adsorption capacity as the ionic strength increases and it can be attributed to the repulsive

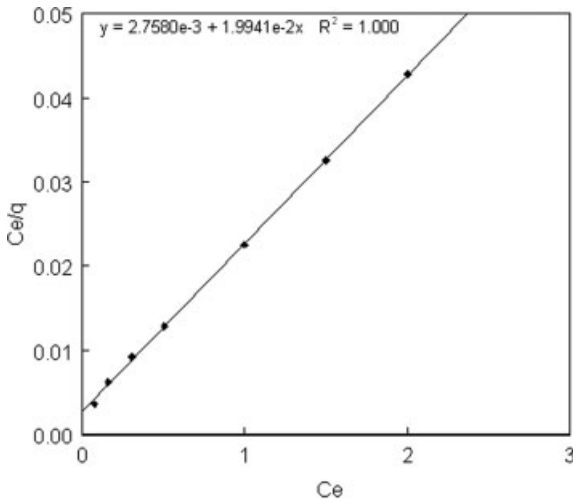


Figure 4 Linear representation of Langmuir equation of IgG; Buffer: 25 m*M* MOPS buffer at pH 6.5; MAH content: 70 μmol/g; *T*: 25°C.

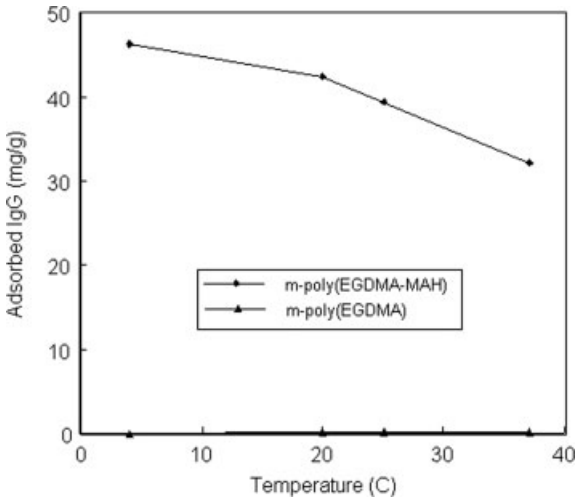


Figure 6 Effect of temperature on IgG adsorption capacity: MAH content: 70 μmol/g; IgG concentration: 0.5 mg/mL; Buffer: MOPS; pH: 6.5.

electrostatic forces between the m-poly(EGDMA-MAH) beads and protein molecules. When the salt concentration increases in the adsorption medium, this can lead to coordination of the imidazole nitrogen atoms of the MAH groups with the salt (NaCl), which leads to low protein adsorption. The distortion of existing salt bridges in the presence of salt also may have contributed to this low protein adsorption at high ionic strength. This adsorption behavior also indicated a mechanism of recognition based on ion-pair formation between the ligand, i.e., MAH and IgG. However, secondary forces such as hydrogen bonding and hydrophobicity cannot be excluded.

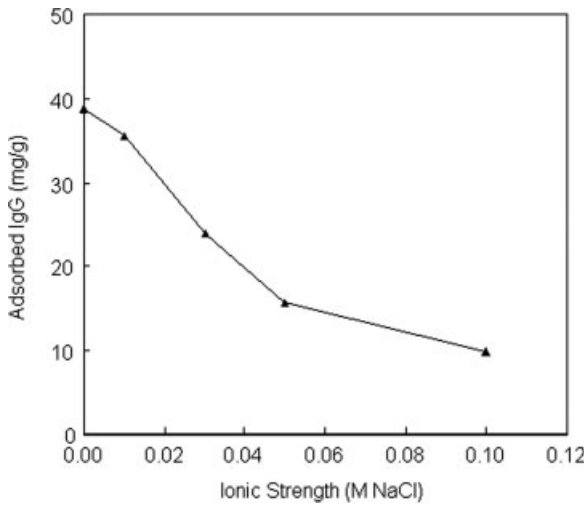


Figure 5 Effect of ionic strength on IgG adsorption. MAH content: 70 μmol/g; IgG concentration: 0.5 mg/mL; Buffer: MOPS; pH: 6.5; *T*: 25°C.

Effect of temperature

The effect of temperature on IgG adsorption was studied in the range of 4–37°C. No significant effect of the temperature was observed on the physical adsorption of the IgG onto the m-poly(EGDMA). However, the equilibrium adsorption of IgG onto the m-poly(EGDMA-MAH) significantly decreased with increasing temperature and the maximum adsorption was achieved at 4°C (Fig. 6). From 5 to 37°C, the adsorption capacity of the magnetic beads decreased for about 30%.

Elution and regeneration of beads

Elution of IgG from m-poly(EGDMA-MAH) beads was also carried out in batch system using 1*M* NaCl at pH 4.0 in acetate buffer. The beads adsorbed with the different amounts of IgG were placed within the adsorption medium, and the amount of IgG eluted in 1 h was determined. The higher elution ratios were obtained. These results may be contributed to elution medium containing 1.0*M* NaCl at pH 4.0 in acetate buffer that might change the charge of the peptide side groups, due to their isoelectric points, resulting in the release of the IgG molecules from the matrix. Elution of IgG is achieved under relatively mild conditions (pH 4.0) compared to conditions employed during affinity purification on protein A sorbents.

A critical issue in affinity chromatography is the number of cycles or reuses that can be achieved. This is due to the high cost of biological ligands. In order to show the reuses of the m-poly(EGDMA-MAH) beads, the adsorption–elution cycle was repeated 10 times using the same magnetic beads. For sterilization, after each adsorption–elution cycle,

the magnetic beads were washed with 50 m*M* NaOH solution for 30 min. After this procedure, the beads were washed with distilled water for 30 min, and then equilibrated with the phosphate buffer for the next adsorption–elution cycle. IgG adsorption capacity decreased 12.4% after 10 cycles using 1.0*M* NaCl at pH 4.0 in acetate buffer (Fig. 7).

IgG removal from serum

Depletion of additional abundant proteins can be beneficial in the analysis of serum proteins, and therefore we attempted to deplete the IgG class immunoglobulins from human serum. In the first step of this study, the depletion of HSA was achieved by using anti-HSA antibody-sepharose 4B column. The depletion efficiency for HSA was 99.2% in serum sample. Then, in the second step, the depletion of IgG from human serum was performed with pseudospecific magnetic beads in batch system. The depletion efficiencies for IgG were above 90% for all studied concentrations. Results, shown in Table II, indicate that a large portion of the IgG was bound by the pseudospecific magnetic beads. To test the efficiency of IgG depletion from human serum, proteins in the serum and eluted portion were analyzed by 2DE. Proteins that were eluted from the pseudospecific magnetic beads include IgG and a small number of nonalbumin proteins. A small number of relatively abundant proteins such as apolipoprotein A1, serotransferrin, haptoglobulin, and α1-antitrypsin were bound by the pseudospecific magnetic beads. We reached up to 98.4% IgG depletion amount, and it may be concluded that pseudospecific magnetic beads are sufficient in terms of efficiency of IgG depletion.

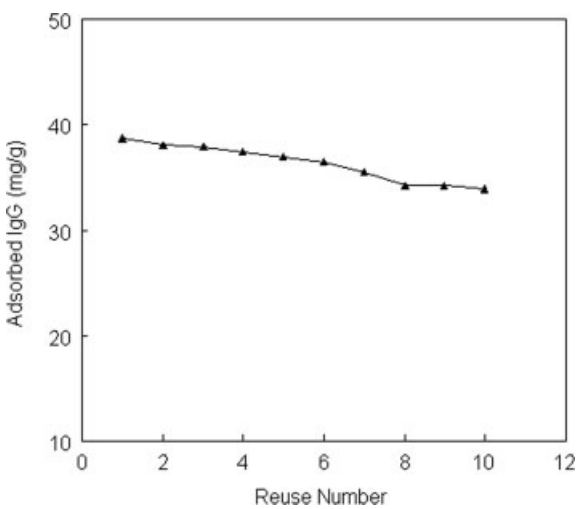


Figure 7 Reusability of m-poly(EGDMA-MAH) beads. MAH content: 70 μmol/g; IgG concentration: 0.5 mg/mL; Buffer: MOPS; pH: 6.5; *T*: 25°C.

TABLE II
IgG Depletion on m-Poly(EGDMA-MAH) Beads from Human Serum

Dilution agent	IgG concentration (mg/ml)	Adsorption amount (mg/g)
Serum (undiluted)	14.5	85.7 ± 2
1/2 diluted serum (phosphate pH: 6.5)	7.0	56.4 ± 2
1/5 diluted serum (phosphate pH: 6.5)	3.5	43.1 ± 2
1/10 diluted serum (phosphate pH: 6.5)	1.8	28.5 ± 2

These results are consistent with published studies.[33,34] Björhal et al. used five different commercially available depletion columns, including Aurum Serum Protein Minikit (Bio-Rad, Hercules, CA), ProteoExtract Albumin/IgG Removal kit (Merck, Darmstadt, Germany), Multiple Affinity Removal Column (Agilent Technologies, San Diego, CA), POROS Affinity Depletion Cartridges (Applied Biosystems, Framingham, MA) and Albumin-IgG Removal Kit (Amersham Biosciences, Uppsala, Sweden).[7] It should be noted that Aurum Serum Protein Minikit (Bio-Rad) and ProteoExtract Albumin/IgG Removal kit contained Protein A as ligand, Multiple Affinity Removal Column and Albumin-IgG Removal Kit contained polyclonal antibodies as ligand. POROS Affinity Depletion Cartridges contained protein G. The depletion efficiencies were above 90%, but due to the high dilution factor after the depletion procedure as compared to crude serum, the concentration of remaining IgG in depleted serum samples were below the detection limits for almost all samples. However, this stated a minimum depletion of 94% of IgG in depleted serum samples by any affinity column. Sitnikov et al. applied Multiple Affinity Removal Column (Agilent Technologies) for the depletion of blood plasma proteins under volatile conditions. The percentage of IgG depletion was greater than 99%.[35] We reached up to 98.4% IgG depletion amount, and it was concluded that the pseudospecific m-poly(EGDMA-MAH) beads offered the promising approach with good depletion specificity and efficiency of IgG.

CONCLUSIONS

The serum proteome has been shown to contain information that directly reflects pathophysiological states and represents an invaluable source of diagnostic information for a variety of diseases. Unfortunately, the dynamic range of protein abundance renders complete characterization of this proteome nearly impossible with current analytical methods.[36] To study low abundance proteins, which have potential value for clinical diagnosis, the high abundant species, such as albumin and immunoglobulins, are generally eliminated as the

first step in many analytical protocols. But, removal of albumin and immunoglobulins from serum is problematic because of its extremely high concentration.[6] Monoclonal antibodies and Protein A/G to IgG are available from many commercial suppliers. These bioligands are extremely specific. But, they are very expensive, because of high cost of production and/or extensive purification steps. In the process of the preparation of specific adsorbents, it is difficult to immobilize antibodies on the supporting matrix with proper orientation.[37–39] Bioligands are also sensitive to the process operating conditions. They can lose activity or leach into the products by the harsh elution and cleaning conditions commonly used in the separation processes. The time consumption and high cost of ligand immobilization procedure has inspired a search for suitable low-cost adsorbents. In this study, novel *N*-methacryloyl-(L)-histidinemethylester (MAH) containing magnetic beads for the affinity depletion of IgG from human plasma were prepared. This approach for the preparation of pseudospecific affinity adsorbent has many advantages over conventional adsorbents. An expensive and critical step in the preparation process of affinity adsorbent is immobilization of an affinity bioligand to the matrix. In this procedure, comonomer MAH acted as the pseudospecific ligand, and there is no need to activate the matrix for the ligand immobilization. Ligand immobilization step was also eliminated in this approach. Another major issue is that of slow release of this covalently bonded ligands off the matrix. Ligand release is a general problem encountered in any affinity adsorption technique which caused a decrease in adsorption capacity. Ligand leakage from the adsorbent causes contaminations that will interfere with analysis of the purified biomolecule. MAH was polymerized with EGDMA and there is no ligand leakage. m-Poly(EGDMA-MAH) beads were also cheap, and resistant to harsh chemicals and high temperatures. These results are encouraging and suggest that further studies should be done to develop magnetic adsorbents.

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