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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Online publication date: 23 April 2002

To cite this Article Özkara, Serpil , Yavuz, Handan , Patır, Süleyman , Arıca, M. Yakup and Denizli, Adil(2002) 'Separation of human-immunoglobulin-G from human plasma with l-histidine immobilized pseudo-specific bioaffinity adsorbents', Separation Science and Technology, 37: 3, 717 — 731
To link to this Article: DOI: 10.1081/SS-120001456
URL: http://dx.doi.org/10.1081/SS-120001456

SEPARATION SCIENCE AND TECHNOLOGY, 37(3), 717–731 (2002)

SEPARATION OF HUMAN-IMMUNOGLOBULIN-G FROM HUMAN PLASMA WITH L-HISTIDINE IMMOBILIZED PSEUDO-SPECIFIC BIOAFFINITY ADSORBENTS

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ABSTRACT

The pseudo-biospecific *affinity* ligand L-histidine immobilized poly(2-hydroxyethylmethacrylate) (PHEMA) in spherical form (100–150 μm in diameter) was used for the affinity chromatographic separation of human-immunoglobulin-G (HIgG) from aqueous solutions and human plasma. The PHEMA adsorbents were prepared by a *radical* suspension polymerization technique. *Reactive aminoacid-ligand* L-histidine was then immobilized by covalent binding onto these adsorbents. Elemental analysis of immobilized L-histidine for nitrogen was estimated as 62.3 mg L-histidine/g of

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PHEMA. The maximum HIgG adsorption on the L-histidine immobilized PHEMA adsorbents was observed at pH 7.4. The non-specific HIgG adsorption onto the plain PHEMA adsorbents was *very* low (about 0.167 mg/g). Higher adsorption values (up to 3.5 mg/g) were obtained when the L-histidine immobilized PHEMA adsorbents were used from aqueous solutions. Much higher amounts of HIgG were adsorbed from human plasma (up to 44.8 mg/g). Adsorption capacities of other blood proteins were obtained as 2.2 mg/g for fibrinogen and 2.8 mg/g for albumin. The total protein adsorption was determined as 52.1 mg/g. The affinity microbeads allowed the one-step separation of HIgG from human plasma. The HIgG molecules could be repeatedly adsorbed and desorbed with these L-histidine-immobilized PHEMA adsorbents without noticeable loss in their HIgG adsorption capacity.

INTRODUCTION

Monoclonol antibodies (i.e., immunoglobulins) play a dual role in the biotechnology industry. They offer exciting potential as diagnostic and therapeutic substances and also serve as bioaffinity ligands for purifying other high-value proteins of pharmaceutical importance such as cytokines and blood-clotting factors (1). Protein A affinity chromatography is a well-known and popular method for purifying immunoglobulins (2). Protein A binds with different affinity to the Fc region of immunoglobulins from a variety of sources, e.g., it binds IgG from human, rabbit, and pig with high affinity, binds horse and cow IgG with lower affinity, and binds rat IgG only very weakly (3). It exhibits a very high specificity and therefore can be employed as a one-step procedure for the purification of antibodies. Due to this specificity, protein A affinity chromatography is now commonly used on a large scale for the purification of immunoglobulins to be used in clinical tests and/or therapy (4). However, in spite of its high selectivity, protein A affinity chromatography also has some drawbacks which are worth considering: (i) a considerable amount of protein A may leak from the chromatography matrix and such contamination cannot, of course, be tolerated in clinical applications; and (ii) the cost of these materials tends to be very high (4). In addition, these types of ligands such as protein A or G are difficult to immobilize in the proper orientation. They are also susceptible to degradation.

On the other hand, pseudo-biospecific ligands can be used to purify a wide range of biomolecules, thus offering more flexibility as compared with biospecific ligands (5). Pseudospecific ligand histidine, which interacts through its carboxyl, amino, and imidazole groups with several proteins at or around their isoelectric points, has shown particular efficacy in separating IgG subclasses from human plasma and purification of monoclonal antibodies from cell culture or ascites fluids

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(6,7). Small aminoacid-molecules may hold certain advantages as ligands for industrial bioaffinity separations since they are not likely to cause an immune response in case of leakage into the product. Small aminoacid-ligands are also much more stable than protein ligands because they do not require a specific tertiary structure for maintaining biological activity (8).

This work explores the performance of L-histidine affinity adsorbents for human-IgG purification from human plasma. The purification of IgG is required generally for the purposes of immunodiagnostics, immunoaffinity chromatography, and immuno-therapy. Moreover, IgG removal from human plasma is employed for the treatment of immune disorders, alloimmunization, and cancer (9). For this reason, clinical application of L-histidine immobilized adsorbents could be a potentially attractive tool (10). In this study, hydrophilic and blood-compatible poly(2-hydroxyethylmethacrylate) (PHEMA) adsorbents were produced in spherical form by a radical suspension-polymerization technique. The hydroxyl groups on these adsorbents were activated and then L-histidine molecules were covalently coupled to the adsorbents through the active sites. Human-immunoglobulin-G (HIgG) adsorption on the L-histidine immobilized PHEMA adsorbents from aqueous solutions containing different amounts of HIgG and at different pH, and also from human plasma are reported here.

EXPERIMENTAL

Preparation of Poly(2-hydroxyethylmethacrylate) Adsorbents

2-Hydroxyethylmethacrylate (HEMA) was purchased from Sigma (St. Louis, MO, USA), and was purified by vacuum distillation under a nitrogen atmosphere. Ethyleneglycoldimethacrylate (EGDMA, Merck, Darmstadt, Germany) was used as the cross-linking agent. 2,2′-Azobisisobutyronitrile (AIBN, BDH, Poole, UK) was used as initiator. The dispersion medium was a saturated aqueous solution of magnesium oxide (MgO) (Sigma).

Poly(2-hydroxyethylmethacrylate) adsorbents were prepared by a radical suspension polymerization technique (11). Polymerization was carried out in an aqueous dispersion medium containing magnesium oxide, which was used to decrease the solubility of the monomer and HEMA in the medium. The monomer phase containing HEMA, EGDMA, and AIBN was added to the dispersion medium within a laboratory type reactor (i.e., a two neck flask with a volume of 500 mL) provided with a blade type stirrer. In order to produce *spherical* adsorbents of about 100–150 μm in diameter and with a narrow size distribution, the HEMA/EGDMA ratio, the monomer phase/dispersion phase ratio, the amounts of EGDMA and AIBN, and the agitation speed were 1:3 (v:v), 1:10 (v/v), 0.33 (mol EGDMA/mol HEMA), 0.0015 (mol AIBN/ mol HEMA), 600 rpm, respectively.

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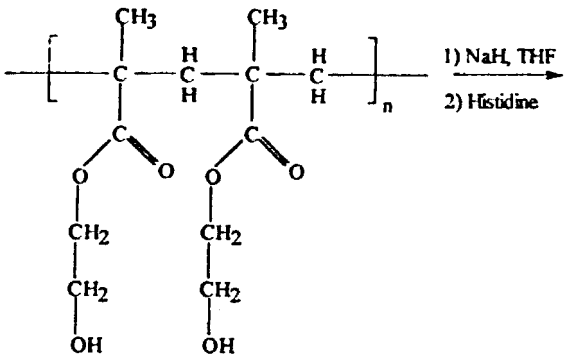
Polymerization was carried out at 70°C for 3 hr and then at 90°C for 1 hr. After cooling, the adsorbents were separated from the polymerization medium by filtration, and the residues (e.g., monomer, MgO, etc.,) were removed by a cleaning procedure (12). Briefly, adsorbents were transferred into a reservoir, and washing solutions (i.e., a dilute HCl solution and a water–ethanol mixture) were recirculated through the system that also includes an activated carbon column, until it was assured that the adsorbents are clean. Purities of the adsorbents were followed by observing the change of optical densities of the samples taken from the liquid phase in the recirculation system, and also from the DSC thermograms of the adsorbents obtained by using a differential scanning microcalorimeter (Mettler, Geneva, Switzerland). Optical density of uncleaned adsorbent was 2.86. But after cleaning operation this value was reduced to 0.06. In addition, when the thermogram of uncleaned adsorbent was recorded, it has a peak around 60°C. This peak might have originated from AIBN. But after application of this cleaning procedure, no peak was observed between 30–100°C on this thermogram.

L-Histidine Immobilization

Pseudospecific affinity-ligand L-histidine was purchased from Aldrich (St. Louis, MO). In order to prepare the L-histidine immobilized PHEMA adsorbents, the following procedure was used. Twenty grams of dry adsorbent was weighed and transferred into the L-histidine solution mixture (3.0 g L-histidine/50 mL tetrahydrofuran) containing 1.4 g of NaH as catalyst. This immobilization reaction was carried out under a constant gentle magnetic stirring (250 rpm) at 40°C for 24 hr. At the end of this reaction period, the L-histidine-immobilized adsorbents were removed by filtration and washed extensively with methanol and water in order to remove weakly adsorbed L-histidine molecules and then dried in vacuum for 24 hr. Chemical immobilization reaction between the adsorbents and L-histidine is as follows:



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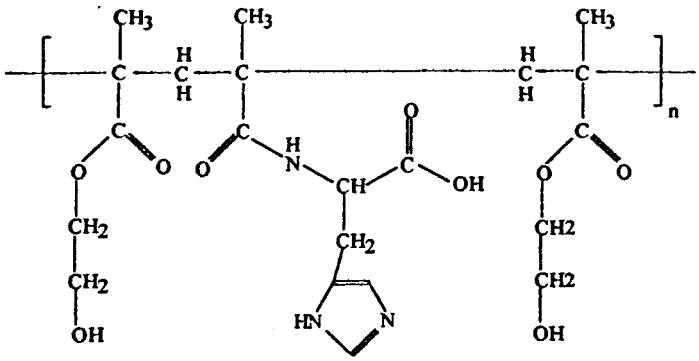

The amount of L-histidine immobilized on the PHEMA adsorbents was determined by measuring the nitrogen concentration in the polymer structure with elemental analysis (LECO, CHNS-932, Chicago, IL).

When not in use, the resulting adsorbents were kept under refrigeration in 0.02% NaN_3 solution to prevent microbial contamination.

The leakage of the L-histidine from the adsorbents was followed by incubating the fully wetted adsorbents with 10 mL of phosphate buffered saline (PBS, pH 7.4) solution for 24 hr at room temperature. The leakage experiments were carried out at 25°C, at a stirring rate of 50 rpm. L-Histidine released after this incubation was measured in the liquid phase spectrophotometrically.

Human-Immunoglobulin-G Adsorption from Aqueous Solution

Adsorption of HIgG (Sigma, Cat. No: 160101) on the L-histidine immobilized poly(2-hydroxyethylmethacrylate) adsorbents was studied batch wise. The PHEMA adsorbents containing L-histidine were incubated with 50 mL of HIgG solution at 20°C for 2 hr (i.e., equilibrium time).

In order to study the effects of pH on coupling of HIgG to L-histidine immobilized PHEMA adsorbents, the pH of the solution was varied between 4.0–10.0 by using different buffer systems (0.1 *M* CH_3COONa–CH_3COOH for pH 4.0–6.0, 0.1 *M* K_2HPO_4–KH_2PO_4 for pH 7.0–8.0 and 0.1 *M* NH_4OH–NH_4Cl for pH 9.0–10.0). The initial concentration of HIgG was 1.50 mg/mL solution.

To observe the effects of the initial concentration of HIgG on adsorption, the initial concentration of HIgG was changed between 0.05 and 5.0 mg/mL.

After the HIgG adsorption, in order to remove the non-specifically adsorbed HIgG molecules, the PHEMA adsorbents 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

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measured using Folin–Lowry method (13). The protein sample (200–400 μL) was diluted to 1.0 mL with PBS. Thereafter 1.0 mL of 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 200–400 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 known concentrations of bovine serum albumin to relate the protein concentration in the solution with the absorbance of the sample. The amount of adsorbed HIgG was calculated as:

$$q = [(C_i - C_t)V]/m \quad (1)$$

where q is the amount of HIgG adsorbed onto unit mass of the adsorbent (mg/g); C_i and C_t are the concentrations of the HIgG in the initial solution and in the supernatant after adsorption, respectively (mg/mL); V is the volume of the aqueous phase (mL); and m is the mass of the adsorbents (g).

Human-Immunoglobulin-G Adsorption from Human Plasma

Adsorption of HIgG from human plasma on the L-histidine immobilized PHEMA adsorbents was studied batch wise. Fresh human blood was used in all experiments. The blood samples (500 mL) were supplied from a healthy donor at the University Hospital (Hacettepe, Ankara). Blood samples were centrifuged at $1000 \times g$ for 30 min at room temperature to separate plasma. Poly(2-hydroxyethyl-methacrylate) adsorbents containing L-histidine were incubated at 20°C for 20 min with 2 mL of human plasma. Phosphate buffered saline (pH: 7.4, NaCl: 0.9%) was used for dilution of human plasma.

The amount of HIgG adsorbed through L-histidine on the PHEMA adsorbents was determined by a solid-phase-enzyme-linked immunosorbent assay method (ELISA) (14). Human anti-IgG (Sigma, I-9384) diluted to 1/1000 in 50 mM $NaHCO_3$, pH 9.6 was adsorbed to PVC microtitre plates at 4°C for 12 hr. The plates were washed with PBS containing 0.05% Tween 20 (Sigma) (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 HIgG were added and incubated at 37°C for 1 hr. Bound HIgG was detected with the anti-HIgG labeled with biotin (Sigma, B-3773) followed by peroxidase-conjugated streptavidin (Sigma) and *o*-phenylenediamine. The absorbance was measured at 492 nm.

In order to show L-histidine specificity, adsorptions of blood proteins (i.e., albumin, fibrinogen, and immunoglobulin-G) were also studied. The L-histidine

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immobilized adsorbents were incubated with a human plasma containing albumin (41.0 mg/mL), fibrinogen (2.4 mg/mL), and γ-globulin (16.8 mg/mL) at room temperature for 2 hr. Total protein concentration was measured by using the total protein reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, England; Catalog Ref. No: 712076) at 540 nm, which was based on Biuret reaction. Chronometric determination of fibrinogen according to the Clauss method on plasma was performed by using Fibrinogene-Kit (Ref No: 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France). Human serum-albumin concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, England; Catalog Ref. No: 229241), which was based on bromocresol green (BCG) dye method. The HIgG concentration was determined by ELISA.

Desorption and Repeated Use

Desorption of HIgG was studied in 2 *M* NaCl aqueous solution. The HIgG-adsorbed PHEMA adsorbents were placed in this desorption medium and stirred continuously (at a magnetic stirring rate of 600 rpm) for 1 hr at room temperature. The total volume of desorption medium was 50 mL. The final HIgG concentration in the desorption medium was determined by ELISA. The desorption ratio was calculated from the amount of HIgG adsorbed on the adsorbents and the final HIgG concentration in the desorption medium.

In order to test the reusability of the L-histidine immobilized PHEMA adsorbents, HIgG adsorption–desorption procedure was repeated for 10 times by using the same polymeric adsorbent. In addition, it should be noted that, after desorption of HIgG, L-histidine leakage from the polymeric structure also was monitored continuously.

RESULTS AND DISCUSSION

L-histidine immobilized PHEMA adsorbents were prepared as a pseudo-specific affinity adsorbent for separation of immunoglobulin-G from the human plasma. The main criteria for selection of PHEMA are its physiological acceptability, mechanical strength, chemical and biological stability, low non-specific adsorption of proteins, and good-blood-compatibility. The observations concluded that PHEMA adsorbents used in this study are resistant to adhesion of blood proteins and blood cells (15). The PHEMA adsorbents prepared in this study are rather hydrophilic and cross-linked structures, i.e., hydrogels. The simple incorporation of water weakens the secondary bonds within the hydrogels. This increases the distance between the polymer chains and causes uptake of

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water. The equilibrium water-uptake ratio of PHEMA adsorbents is 55% (w/w). The aqueous swelling properties of the PHEMA adsorbents were not observed to change following derivatisation with L-histidine.

L-Histidine molecules were immobilized covalently to the PHEMA adsorbents. Histidine coupled to the polymer adsorbents via its amino group; the carboxyl group and imidazole ring remain free.

It is accepted that amide bonds are formed between the amino groups of the L-histidine and the carbonyl groups of the PHEMA. L-histidine leakage also was investigated in PBS solution. Leakage was not observed from the L-histidine-immobilized adsorbents.

Elemental analysis of the plain PHEMA and the L-histidine-immobilized PHEMA adsorbents was performed, and the attachment of the L-histidine was found as 62.3 mg/g from the nitrogen stoichiometry.

Human-Immunoglobulin-G Adsorption from Aqueous Solutions

In this study, the effects of medium pH and initial concentration of HIgG in the incubation medium on the adsorption of HIgG molecules onto the plain and the L-histidine immobilized PHEMA adsorbents were studied in batch experiments.

Figure 1 shows the effects of pH on HIgG adsorption, which is very significant. As shown in Fig. 1, the maximum HIgG-binding capacity of affinity adsorbents was found to lie at 2.4 mg HIgG/g, which was observed at pH 7.0. Significant lower adsorption capacities were obtained below and above pH 7.0. The amount of HIgG coupled onto the L-histidine-immobilized PHEMA adsorbents as a function of pH exhibits two adsorption domains, as shown in Fig. 1. HIgG is negatively charged at pH 7.0 (isoelectric point of HIgG: 6.2). But it is interesting to note that the amount of HIgG coupled onto L-histidine containing adsorbents shows a maximum at pH 7.0, with a very significant decrease at lower and higher pH values. Pseudo-specific interactions between HIgG and aminoacid-ligand L-histidine molecules at pH 7.0 may resulted from the conformational state of HIgG molecules (more folded structure) at this pH (pKa value of histidine is around 6.5). It should be noted also that non-specific HIgG adsorption on plain PHEMA adsorbent is independent of pH and it is observed at the same at all the pH values studied.

Figure 2 gives the adsorption data on the plain and L-histidine immobilized PHEMA adsorbents (0.167 mg HIgG/g). Pseudo-specific adsorption (i.e., adsorption of HIgG molecules onto the PHEMA adsorbents through L-histidine molecules) was significant (up to 3.5 HIgG/g) and increased with increase in the initial concentration of HIgG in the incubation medium. As expected, the amount of HIgG coupled to PHEMA adsorbents via L-histidine molecules reached almost a plateau value around 4.0 mg/mL, due to the saturation of active binding sites.

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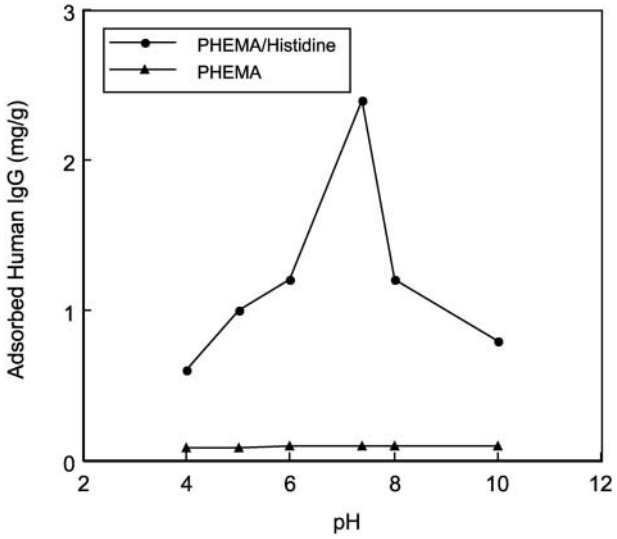

Figure 1. Effect of pH on human-IgG adsorption on L-histidine immobilized onto PHEMA adsorbents; L-histidine loading: 62.3 mg/g; Human-IgG concentration: 1.5 mg/mL; *T*: 25°C.

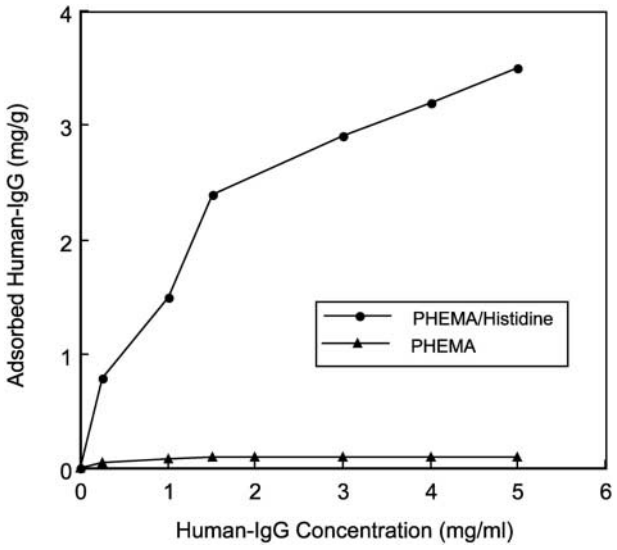

Figure 2. Effect of human-IgG initial concentration on human-IgG adsorption through L-histidine immobilized onto PHEMA adsorbents; L-histidine loading: 62.3 mg/g; pH: 7.4; *T*: 25°C.

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Immobilized L-histidine also proved useful for the capture of HIgG directly from healthy human plasma. The IgG molecules were found to be adsorbed on immobilized L-histidine via their Fab part (16). Figure 3 gives the adsorption data. A negligible amount of protein adsorbed non-selectively on the adsorbents is one of the most important requisites. Hydrated water on the hydrophilic polymer surface will reduce the hydrophobic interaction between the protein molecules and the polymer surface. As seen here, very low non-specific adsorption of HIgG onto the plain PHEMA adsorbents was obtained. However, there was a pronounced adsorption of HIgG (up to 44.8 mg/g) from human plasma onto the L-histidine immobilized PHEMA adsorbents. The adsorption of HIgG onto the PHEMA adsorbents containing 62.3 mg L-histidine/g from human plasma was approximately 12-fold higher than that obtained in the studies where aqueous solutions were used. This is due to the high initial concentration of HIgG in the plasma. Human immnuoglobulin-G has a molecular mass of 150,000 and consists of four peptide chains; two identical light chains. These chains are linked by strong disulphide bonds into a Y- or T-shaped structure with hinge-like flexible arms. Thus an IgG molecule would expand and contract significantly with the variation of the ionizable groups in the molecule. This high HIgG adsorption may be also due to suitable conformation of HIgG molecules within their native medium (i.e., human plasma pH 7.4) for interaction with L-histidine molecules onto the PHEMA adsorbents.

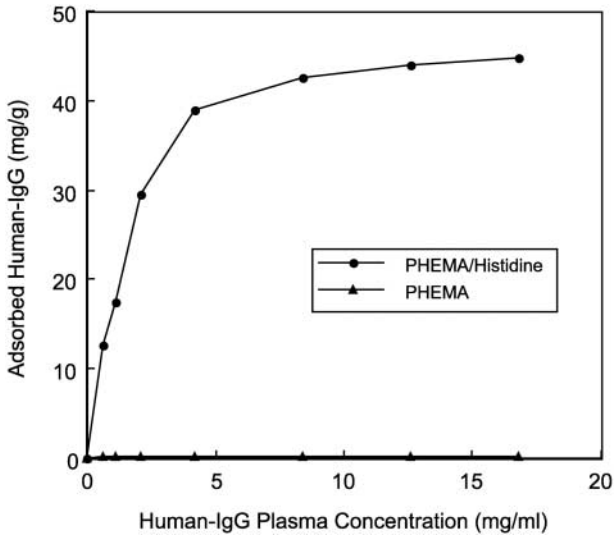

Figure 3. Human-IgG adsorption from human plasma through L-histidine immobilized onto PHEMA adsorbents; L-histidine loading: 62.3 mg/g; *T*: 25°C.

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In order to show the L-histidine specificity, adsorption of other blood proteins also was studied. Interesting results were obtained in these studies. Adsorption capacities were obtained as 44.8 mg/g for HIgG, 2.2 mg/g for fibrinogen, and 2.8 mg/g for albumin. The total protein adsorption was determined as 52.1 mg/g. It is worth noting that adsorption of other plasma proteins (i.e., fibrinogen and albumin) on the L-histidine immobilized adsorbents are negligible. It may be concluded that this low adsorption of fibrinogen and albumin is due to the high affinity of L-histidine towards HIgG molecules.

Comparison with Related Literature

Note that different affinity sorbents with different adsorption capacities were reported in literature for human-IgG adsorption. Füglistaller was interested in determining the dynamic binding capacities of different commercial protein A affinity chromatography matrices including Affi-Gel, Eupergit, Ultrogel, Sepharose series, and Prosep A (4). He presented adsorption capacities of 0.7–20.1 mg IgG_3/g. Klein et al. used microporous poly(caprolactam) hollow fibers and flat sheet membranes as the carrier matrix, and immobilized recombinant protein A as specific bioligand. They reported human-IgG adsorption capacities around 12.4–28.3 mg/cm^3 (17). Kim et al. used hydrophobic amino acids (e.g., phenylalanine and tryptophan) containing membrane based on polyethylene and they obtained 50 mg/g polymer for bovine gamma globulin (18,19). Muller-Shulte et al. used several polymeric carriers made of different polymers including Biograft, Sepharose 4B, Superose and Spherosil, and histidine as the pseudo-specific ligand (20). Their maximum IgG_1 adsorption values were in the range of 0.05–0.23 mg IgG_1 per milliliter sorbent. Bueno et al. used Poly(ethylene vinyl alcohol) hollow fiber cartridge carrying L-histidine and they reported dynamic adsorption values up to 77.7 ± 2.7 mg IgG per gram polymer (21). Denizli and Pişkin reported 24.0 mg HIgG/g adsorption capacity with protein A-immobilized-poly(2-hydroxyethylmethacrylate) (22). Charcosset et al. used microporous immunoaffinity hollow fibers composed of polysulfone/protein A and they obtained 8.8 ± 0.4 mg/g adsorption capacity (23). Langotz and Kroner reported 0.51 mg/mL rabbit immunoglobulin G adsorption capacity with commercially available Sartobind Epoxy adsorbents (24). Dancette et al. explored the performance of recombinant protein A/G affinity membranes based on polymethylmethacrylate and polyacrylonitrile for human and mouse IgG purification and they obtained the static binding capacity as 6.6 mg IgG per mL membrane (25). Castillo et al. coated nylon microfiltration membranes with dextran and poly (vinyl alcohol) to produce affinity matrices. They immobilized protein A to these membranes for the adsorption of HIgG. They achieved adsorption capacities in the range of 7.02–13.28 mg/mL (26). We demonstrated

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that L-histidine carrying PHEMA adsorbents exhibit comparable HIgG binding capacities with affinity adsorbents including commercial media.

Desorption of Human-Immunoglobulin-G

Desorption of HIgG from the L-histidine immobilized PHEMA adsorbents was performed in a batch experimental set-up. The adsorbents loaded with HIgG were placed within the desorption medium and the amount of HIgG desorbed in 1 hr was determined. It should be noted that human plasma was used for repeated human-IgG adsorption cycles. Up to 98% of the adsorbed HIgG was desorbed by using 2.0 *M* NaCl as elution agent. The addition of NaCl might change the charge of the peptide side groups due to their isoelectric points, resulting in the detachment of the IgG molecules from L-histidine. Note that there was no L-histidine release in this case, which shows that L-histidine molecules are immobilized covalently to PHEMA adsorbents.

In order to show the reusability of the L-histidine immobilized PHEMA adsorbents, the adsorption-desorption cycle was repeated for 10 times using the same polymeric adsorbents. There was no remarkable reduce in the adsorption capacity of the adsorbents (Fig. 4). The HIgG adsorption capacity decreased by only 1.0% after 10 cycles. With the desorption data given above, we concluded

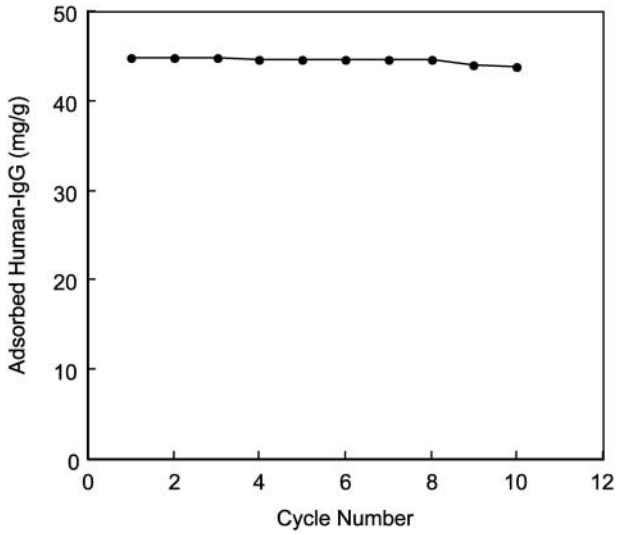

Figure 4. Repeated use of L-histidine immobilized PHEMA adsorbents; L-histidine loading: 62.3 mg/g; *T*: 25°C.

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that NaCl is a suitable desorption agent and allows repeated use of the pseudo-specific affinity sorbents used in this study.

CONCLUSIONS

Immunoglobulins are becoming an important class of biomolecules for both the diagnosis and the treatment of a large variety of human diseases. Immunoglobulins of the G class are finding application for the diagnosis and cure of certain important diseases, such as cancer also (27). The medical and commercial relevance of immunoglobulins have stimulated the development of cost and time effective purification techniques including polymeric carriers. Protein A affinity chromatography can not be used for large scale purification, since they are very expensive. Aminoacid containing adsorbents may hold certain advantages as ligands for industrial affinity separations since they are not likely to cause an immune response in case of leakage into the product (28). Small ligands are also much more stable than antibody ligands because they do not require a specific tertiary structure for maintaining biological activity. They can be manufactured aseptically in large quantities under good manufacturing practices conditions, and at significantly lower cost. In addition, amino acid ligands may be easily modified existing chemical methods to facilitate product elution under mild conditions (29,30).

Poly(2-hydroxyethylmethacrylate) adsorbents were produced by radical suspension-polymerization of HEMA. An aminoacid-ligand, L-histidine was then immobilized to these adsorbents to have a loading up to 62.3 mg/g, which resulted in a HIgG adsorption of 3.5 mg/g from aqueous solutions. A remarkable increase in the HIgG adsorption capacities was achieved from human plasma (up to 44.8 mg/g). Successful desorption ratios (more than 95% of the adsorbed HIgG) were achieved by using 2.0 *M* NaCl. It was possible to reuse these L-histidine immobilized pseudo-specific affinity adsorbents without remarkable reduction in the adsorption capacities.

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Received October 2000
Revised April 2001

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