Porous Dye Affinity Beads for Albumin Separation from Human Plasma

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ABSTRACT: Porous polymeric beads were obtained by the suspension polymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA). Poly(HEMA–EGDMA) beads were characterized by surface area measurements, swelling studies, FTIR, scanning electron microscopy (SEM), and elemental analysis. Poly (HEMA–EGDMA) beads had a specific surface area of 56 m^2/g. SEM observations showed that the poly(HEMA–EGDMA) beads abounded macropores. Poly(HEMA–EGDMA) beads with a swelling ratio of 55%, and containing different amounts of Reactive Red 120 (9.2–39.8 μmol/g) were used in the adsorption/desorption of human serum albumin (HSA) from aqueous solutions and human plasma. The nonspecific adsorption of HSA was very low (0.2 mg/g). The maximum HSA adsorption amount from aqueous solution in phosphate buffer was 60.1 mg/g at pH 5.0. Higher HSA adsorption value was obtained from human plasma (up to 95.7 mg/g) with a purity of 88%. The equilibrium monolayer adsorption amount, Q_{max} was determined as 172.4 mg/g. The dimensionless separation factor (R_L) value shows that the adsorption behavior of HSA onto the Reactive Red 120 attached poly(HEMA–EGDMA) beads was favorable ($0 < R_L < 1$). Desorption of HSA from Reactive Red 120 attached poly (HEMA–EGDMA) beads was performed using 0.1*M* Tris/HCl buffer containing 0.5*M* NaCl. It was observed that HSA could be repeatedly adsorbed and desorbed with Reactive Red 120-attached poly(HEMA–EGDMA) beads without significant loss in the adsorption amount. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 1251–1260, 2007

Key words: dye-affinity chromatography; porous beads; protein purification; albumin

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in the human circulatory system.[1] It consists of a single, nonglycosylated, polypeptide chain containing 585 amino acid residues and has many physiological functions that contribute significantly to colloid osmotic blood pressure and aid in the transport, distribution, and metabolism of many endogenous and exogenous substances including bile acids, bilirubin, long-chain fatty acids, amino acids (notably tryptophan, tyrosine and cysteine), steroids (progesterone, testosterone, aldosterone, cortisol), metal ions such as copper, zinc, calcium, magnesium, and numerous pharmaceuticals.[2] HSA commonly used for therapeutic purposes such as shock, heavy loss of blood, etc., requires relatively high purity for medical use. Research on HSA separation has attracted considerable attention for its great potential in blood protein manufacture. HSA is at present commonly isolated from human plasma by Cohn's classical blood fractionation procedure.[3] Cohn's method concerns precipitation of proteins using ethanol with varying pH, ionic strength, and temperature. But this technique, which is the oldest method of industrial fractionation of blood proteins, is not highly specific and can give partially denaturated proteins.[4]

Dye-ligand chromatography has been used extensively in laboratory and large scale protein purification.[5–10] Dye-ligands are commercially available, inexpensive, and can easily be immobilized, especially on matrices having hydroxyl groups. Although dyes are all synthetic in nature, they are classified as affinity ligands because they interact with the active sites of many proteins mimicking the structure of the substrates, cofactors, or binding agents for those proteins. A number of textile dyes, known as reactive dyes, have been used for protein purification.[11] Most of these reactive dyes consist of a chromophore (either azo dyes, anthraquinone, or phathalocyanine) linked to a reactive group (often a mono- or dichlorotriazine ring). The interaction between the dye ligand and proteins can be a complex combination of electrostatic, hydrophobic, and hydrogen bonding.[12] There are several methods for attachment of dye molecules onto the support matrix, in which usually several intermediate steps are followed. Both the

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adsorption and elution steps should carefully be optimized and redesigned for a successful separation.

This work reports on the adsorption of an HSA from aqueous solutions and human plasma by dye affinity chromatography with a novel dye-ligand. Poly(HEMA–EGDMA) bead is a copolymer of 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EGDMA), which was obtained by suspension polymerization. Poly(HEMA–EGDMA) beads were characterized using FTIR, scanning electron microscope, porosity measurements, elemental analysis, and swelling test. HSA adsorption on the poly(HEMA–EGDMA) beads from aqueous solutions containing different amounts of HSA, at different ligand contents, pHs and ionic strengths, and also from human plasma was performed. In the last part, desorption of HSA and stability of these materials was tested.

MATERIALS AND METHODS

Chemicals

HEMA (Sigma Chem., St. Louis) and EGDMA (Aldrich, Munich, Germany) were distilled under vacuum (100 mmHg). Reactive Red 120 was also obtained from Sigma and used without further purification. Poly(vinyl alcohol) (MW: 100.000, 98% hydrolyzed) and HSA (98% pure by gel electrophoresis, fatty acid free, 67 kDa) were purchased from Aldrich (Munich, Germany). α-α′-Azobisisobutyronitrile (AIBN) was obtained from Fluka A.G (Buchs, Switzerland), and used as received. All other chemicals were of the highest purity commercially available and were used without further purification. Coomassie Blue for the Bradford Protein assay was supplied from BioRad (Richmond, CA). All water used in the experiments were 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.

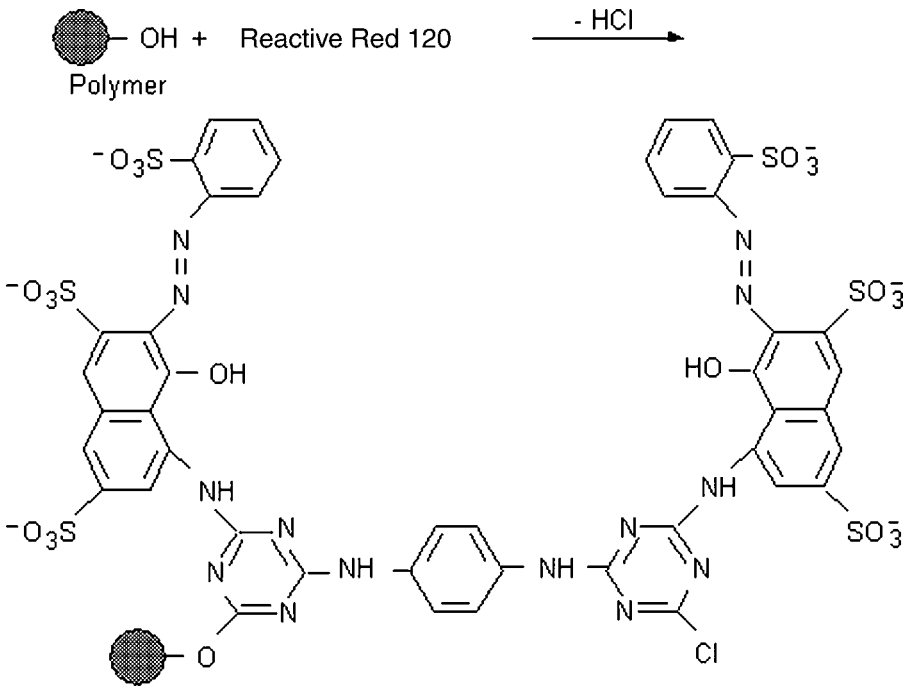
Preparation of the poly(HEMA–EGDMA) beads

A porous crosslinked copolymer was prepared by the reaction of hydroxyethyl methacrylate (HEMA) and EGDMA by the procedure given elsewhere.[13] To obtain polymeric beads with an average diameter of 150–200 μm, the following experimental recipe was used: the dispersion medium was prepared dissolving 200 mg of poly(vinyl alcohol) within 50 mL of distilled water. Then 12 mL toluene was mixed with 4 mL of HEMA and 8 mL of EGDMA, and then 60 mg of AIBN was added. This monomer phase was then transferred into the dispersion medium placed in a glass polymerization reactor (100 mL), which was in a thermostatic water bath. Polymerization medium was magnetically stirred at 600 rpm. The reactor was flushed by bubbling nitrogen and then was sealed. The mixture was reacted at 65°C for 4 h, and then at 90°C for 2 h. The beads were collected by filtration under suction, and washed sequentially with water and ethyl alcohol, and dried in a vacuum oven at 60°C for 48 h.

Dye incorporation to the poly(HEMA–EGDMA) beads

To have the beads carry different amounts of dye, the following procedure was applied: 10 mL of the aqueous solution containing various amounts of Reactive Red 120 (0.25–2.0 mg/mL) was poured into 90 mL of the suspension of the beads in purified water (containing 3.0 g of the beads), and then 4.0 g of NaOH was added. The medium was heated in a sealed reactor for 4 h at 400 rpm and at 80°C. Under these experimental conditions, a nucleophilic substitution reaction took place between the chlorine containing group of the Reactive Red 120 and the hydroxyl groups of the HEMA monomer, with the elimination of NaCl, resulting in covalent attachment of Reactive Red 120 onto the beads. Chemical reaction between Reactive Red 120 and poly(HEMA–EGDMA) beads is shown in Scheme 1. The covalent coupling of Reactive Red 120 to the poly(HEMA–EGDMA) beads results from the formation of an ether linkage between the reactive triazine ring of the dye and the hydroxyl groups of the HEMA. Any remaining chlorine atoms in the dye-attached beads due to the dichloro triazinyl dye structure, after covalent immobilization, was converted to amino groups by treating with 2*M* NH_4Cl at pH 8.5 for 24 h at room temperature. The dye-attached beads were filtered, and washed sequentially with distilled water and methanol several times until all the unbound dye was removed. Reactive Red 120-attached poly(HEMA–EGDMA) beads were stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

The release of Reactive Red 120 from the dye-attached beads was investigated at different pH values in the range of 4.0–8.0. These media were the same ones used in the HSA adsorption experiments described later. The Reactive Red 120 release was also determined in a medium 0.1*M* Tris/HCl buffer containing 0.5*M* NaCl, which was the medium used in the HSA desorption experiments. The medium with the Reactive Red 120 attached poly(HEMA–EGDMA) beads was incubated at room temperature for 24 h. Then, the beads were removed from the medium, and the Reactive Red 120 concentration in



Scheme 1 Chemical reaction between Reactive Red 120 and poly(HEMA–EGDMA) beads.

the supernatant was measured by spectrophotometer at 630 nm.

Characterization of beads

Water uptake ratio of the beads was determined in distilled water. The experiment was conducted as follows: initially dry beads were carefully weighed before being placed in a 50 mL vial containing distilled water. The vial was put into an isothermal water bath at 25°C for 24 h. The beads were taken out from the water, wiped using a filter paper, and weighed. The mass ratio of dry and wet samples was recorded. The water content of the beads was calculated using the following expression:

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

where W_o and W_s are the mass of poly(HEMA–EGDMA) beads before and after uptake of water, respectively.

The morphology of a cross section of the dried beads was investigated by scanning electron microscope (JEOL, JEM 1200 EX, Tokyo, Japan). The beads were dried in a vacuum oven at 50°C for 24 h. 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 (Milano, Italy). The specific surface area of the beads was determined in BET isoterm of nitrogen with an ASAP2000 instrument (Micromeritics). FTIR spectra of the Reactive Red 120, the poly(HEMA–EGDMA) beads, and Reactive Red 120-attached poly(HEMA–EGDMA) beads were obtained by using a FTIR spectrophotometer (FTIR 8000 Series, Shimadzu, Japan). The dry beads (about 0.1 g) were thoroughly mixed with KBr (0.1 g, IR Grade, Merck, Germany), pressed into a tablet, and the spectrum was then recorded. To evaluate Reactive Red 120 content, the poly (HEMA–EGDMA) beads were subjected to elemental analysis using a Leco Elemental Analyzer (Model CHNS-932).

HSA-adsorption from aqueous solutions

The HSA adsorption studies were carried out in a batch system. The beads were washed with 30 mL of water and then equilibrated with 25 m*M* phosphate buffer containing 0.1*M* NaCl (pH 7.4). Then, the prepared HSA solution (50 mL of the aqueous HSA solution) was contacted with the beads in a magnetically stirred reactor for 2 h. The adsorption was followed by monitoring the decrease in UV absorbance at 280 nm. Effects of HSA concentration, pH of the medium, and ionic strength on the adsorption amount were studied. To observe the effects of the initial concentration of HSA on adsorption, it was changed between 0.05–1.0 mg/mL. To determine the effect of pH on the adsorption, pH of the solution was changed between 4.0 and 8.0. To observe the effects of ionic strength, NaCl solution was used at ionic strength values of 0.01 and 0.1.

Desorption and repeated use

In all cases adsorbed HSA molecules were desorbed using 0.1*M* Tris/HCl buffer containing 0.5*M* NaCl. In a typical desorption experiment, 50 mL of the desorption agent was contacted with the protein adsorbed beads for 1 h. The final HSA concentration in the desorption medium was spectroscopically determined. When desorption was achieved, the beads were cleaned with 1*M* NaOH and then re-equilibrated with 25 m*M* phosphate buffer containing 0.1*M* NaCl (pH 7.4). The desorption ratio was calculated from the amount of HSA adsorbed on the beads and the final HSA concentration in the desorption medium. To test the repeated use of poly (HEMA–EGDMA) beads, HSA adsorption–desorption cycle was repeated five times using the same modified beads. To regenerate and sterilize, after the desorption, the modified beads were washed with 1*M* NaOH solution.

HSA-adsorption from human plasma

Human blood was collected into EDTA-containing vacutainers and red blood cells were separated from plasma by centrifugation at 4000 × *g* for 30 min at room temperature, then filtered (3 μm Sartorius filter) and frozen at −20°C. Before use, the plasma was thawed for 1 h at 37°C. Before application, the viscous sample was diluted with 25 m*M* phosphate buffer containing 0.1*M* NaCl (pH 7.4). Dilution ratios were 1/2 and 1/10. 50 mL of the human plasma with a HSA content of 37.7 mg/mL was contacted with the beads for 1 h. HSA concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics, Halstead, Essex, England), which based on bromocresol green dye method.[14] To show dye specificity, adsorption of other blood proteins (i.e., fibrinogen and γ-globulin) was also monitored. Total protein concentration was measured by using the total protein reagent (Ciba Corning Diagnostics, Halstead, Essex, England) at 540 nm which based on Biuret reaction.[14] Chronometric determination of fibrinogen according to the Clauss method on plasma was performed by using Fibrinogene-Kit (Ref No: 68,452 and 68,582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France).[15] γ-globulin concentration was determined from the difference.

The purity of HSA was assayed by sodium dodecylsulfate-polyacrylamide gel electrophoresis using 10% separating gel (9 cm × 7.5 cm) and 6% stacking gels were stained with 0.25% (w/v) Coomassie Brillant R 250 in acetic acid–methanol–water (1 : 5 : 5, v/v/v) and destained in ethanol–acetic acid–water (1 : 4 : 6, v/v/v). Electrophoresis was run for 2 h with a voltage of 110 V. Lysozyme and HSA were used as standards.

Adsorption characteristics

Data obtained from the adsorption experiments of HSA onto the Reactive Red 120-attached poly (HEMA–EGDMA) beads were fitted to the modified empirical Langmuir equation.

$$C_e/Q_e = 1/K_L + (a_L/K_L)C_e \quad (2)$$

where C_e is the equilibrium concentration of HSA, Q_e is the adsorbed amount of HSA, K_L and a_L are isotherm constants for particular solute–solvent combination. The dimensionless separation factor (R_L) indicates the shape of the Langmuir isotherm to be either favorable ($0 < R_L < 1$), unfavorable ($R_L > 1$), linear ($R_L = 1$) or irreversible ($R_L = 0$).

$$R_L = 1/(1 + K_L \times C_o) \quad (3)$$

where C_o is the initial HSA concentration (mg/mL).

RESULTS AND DISCUSSION

Poly(HEMA–EGDMA) (150–250 μm) carrying Reactive Red 120 were prepared as affinity adsorbents for HSA adsorption. Details of the preparation and characterization of poly(HEMA–EGDMA) beads can be found in our previous article.[16] The main selection criteria of poly(HEMA–EGDMA) are its mechanical strength and chemical stability. The hydrophilic poly(HEMA–EGDMA) beads are a crosslinked structure. They do not dissolve in aqueous media, but do swell, depending on the degree of crosslinking. The equilibrium swelling ratio (the ratio of the volumes of the beads before and after swelling) of the beads is 55%. These swollen beads have an average diameter within the range of 150–250 μm. It should be noted that these dye-attached beads were suitable for packed or fluidized bed column applications.

According to the mercury porosimetry data, the pore radii of the poly(HEMA–EGDMA) beads changed between 175 and 400 nm. This indicated that the polymeric beads contained mainly macropores. This pore diameter range was possibly available for the diffusion of the HSA molecules. The molecular size of HSA is 38 nm × 150 nm.[17] On the basis of these data, it can be concluded that the beads had effective pore structures for the liquid chromatographic separation of HSA.

The surface area of the beads was found to be 56 m^2/g by the BET method. After Reactive Red 120 attachment, the specific surface area was found

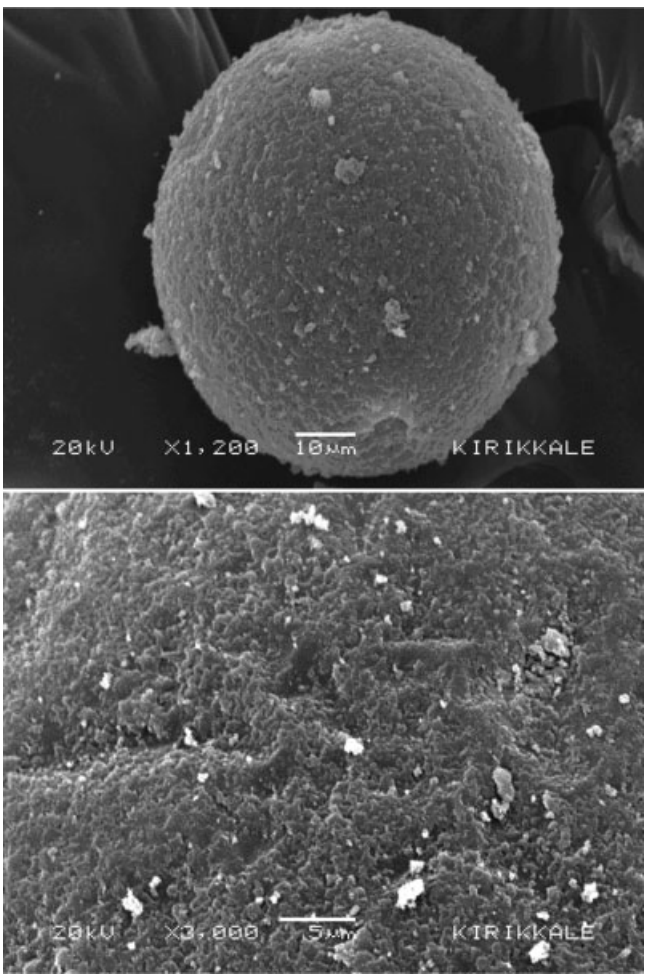


Figure 1 The surface morphology and internal structure of the poly(HEMA–EGDMA) beads.

to be same. Therefore these pores were not blocked by the attached dye molecules.

The surface morphology and internal structure of the poly(HEMA–EGDMA) beads are shown by the electron micrographs in Figure 1, which shows that the beads are spherical and have a rough surface. Figure 1(b) was taken with broken beads so that we could observe the internal part of the polymeric structure. The presence of macropores within the bead interior can be clearly seen in this photograph. It can be concluded that the poly(HEMA–EGDMA) beads had a macroporous interior surrounded by a reasonably rough surface in the dry state. The roughness of the bead surface should be considered as a factor increasing the specific surface area. In addition, these macro pores reduced the diffusional resistance and facilitated mass transfer because of the micro porous structure. This also provided higher Reactive Red 120 attachment and enhanced the HSA adsorption amount.

Poly(HEMA–EGDMA) can be fairly reactive in nucleophilic substitution reaction via free alcoholic-OH groups and occurring chemical bond if substrate has a leaving group which is suitable for substitution reaction, such as chloro atoms. Thus, an adsorbent can be yielded containing aromatic groups, for example dye molecules, especially, Reactive Red 120, which has a lot of aromatic structure. Figure 2 shows FTIR spectra of plain and modified beads. In the spectrum belonging to Reactive Red 120 [Fig. 2(b)], the absorption bands in the range from 1324 to 1617 cm^{-1} correspond to vibration of aryl-C=C bonds. The Reactive Red 120 constituted the origin of two naphthalene rings, three aniline groups, and two triazine rings, and absorption bands in this region is therefore overlap because of high intensity of the absorption bands of their C=C bonds aromatic rings. The broad peak close to 3446 cm^{-1} is indicated by N—H and phenolic-O—H groups. Absorption bands 1203 and 1045 cm^{-1} refer to SO_2 asymmetric stretching and S=O vibrations.

The absorption band of the functional groups of the poly(HEMA–EGDMA) can be clearly seen in Figure 2(c). Absorption bands 3466 and 2987/2955 cm^{-1} are because of the stretching of alcoholic-OH and aliphatic CH_2/CH_3, respectively. The intensive peak 1727 cm^{-1} correspond to C=O group. Peaks 1457 and 1370 cm^{-1} are absorption bands of $(CH_3)_2C$- stretching. C—H out-of-plane stretching absorbs infrared radiation 1263 and 1158 $cm^{-1}$and C—O—C also at 1158 cm^{-1}.

When poly(HEMA–EGDMA) and Reactive Red 120 attached poly(HEMA–EGDMA) spectra are compared with each other, the major differences that can be seen are the disappearance of C—Cl absorption bands and enhanced intensity of 3446 cm^{-1} peak, which refer to N—H, phenol-OH, and nonbonding alcoholic-OH. On the other hand, while the ratio of 3511 cm^{-1} to 2987 or 2955 cm^{-1} peaks is almost equal to one in Figure 2(a), this ratio is bigger than one in Figure 2(c), because N—H and phenol-OH bonds enhance intensity of absorption band.

Reactive Red 120 is a dichlorotriazine dye and it contains six sulfonate groups, four secondary amino groups, and two hydroxyl groups. The strong binding of the dye ligands to proteins may have resulted from a cooperative effect of different mechanisms, such as hydrophobic and ion-exchange interactions caused by the aromatic structures and acidic groups on the dye ligand and by groups on the side chains of amino acids on the protein molecules.[18] The dye ligands are not very hydrophobic overall, but they do have planar aromatic structures that prefer to interact with hydrophobic residues in proteins. The Reactive Red 120 molecules were covalently attached into the poly(HEMA–EGDMA) beads. Reactive Red 120 attachment onto the poly(HEMA–EGDMA) beads was macroscopically detected by the change of color from white to red. It is accepted that ether linkages

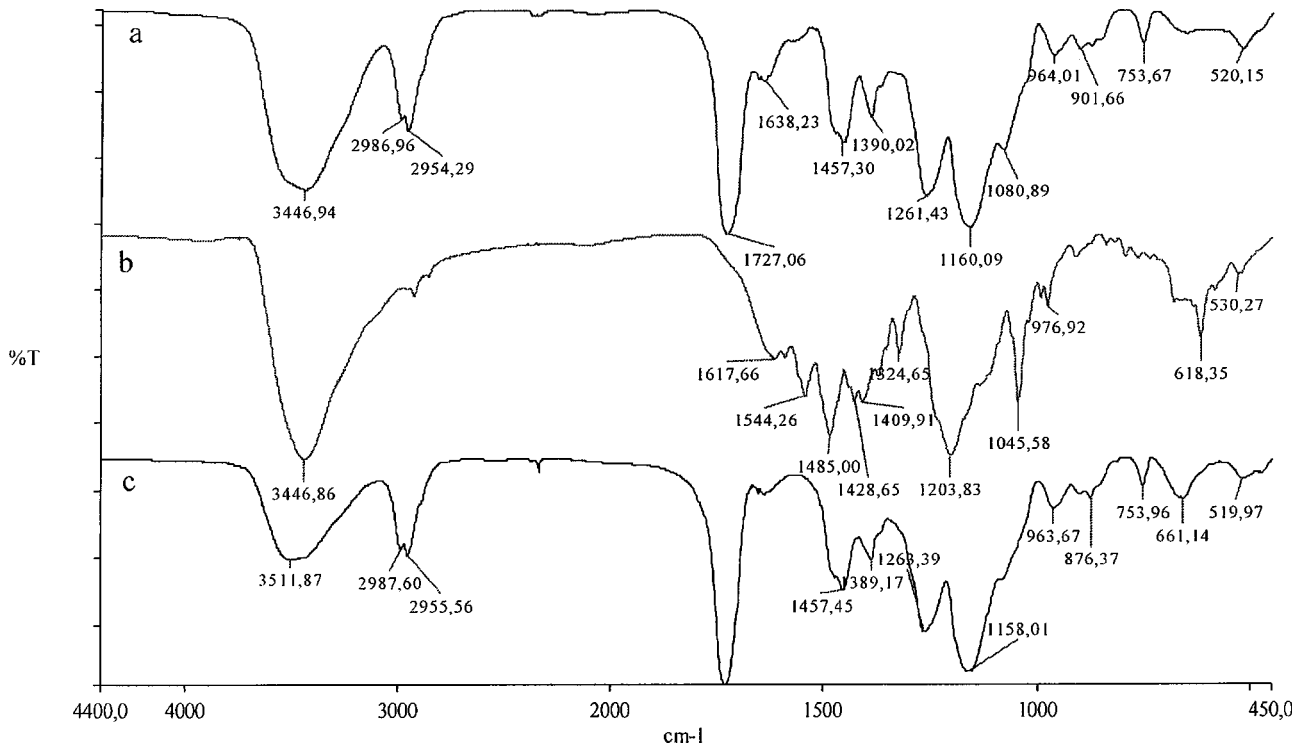


Figure 2 FTIR Spectra of: (a) Reactive Red 120 attached poly(HEMA–EGDMA), (b) Reactive Red 120, and (c) poly (HEMA–EGDMA).

are formed between reactive triazine ring of the dye and the hydroxyl groups of the sorbent. The Reactive Red 120 attached beads were extensively washed to ensure that there was no dye leakage from any of the dye-attached beads and in any media used at adsorption or desorption steps.

HSA adsorption–desorption studies

Effect of the Reactive Red 120 content

The Reactive Red 120 content on the HSA adsorption is shown in Figure 3. As expected, higher levels of HSA adsorption are observed in the more highly Reactive Red 120 loaded poly(HEMA–EGDMA) beads. The HSA adsorption amount increased with increasing Reactive Red 120 content in poly(HEMA–EGDMA) beads up to 25.5 μmol/g. At higher Reactive Red 120 contents, however, the increase in the HSA adsorption amount is slightly small. This decrease is probably because of steric effects, such as Reactive Red 120 coverage and pore blockage by adsorbed HSA molecules. Reactive Red 120 attached poly(HEMA–EGDMA) beads contained 39.8 μmol/g, which is the maximum value reached. The maximum HSA adsorption is 60 mg/g. Over the 25.5 μmol/g Reactive Red 120 content, steric hindrance between the dye and HSA molecule is important, and in this case, all the Reactive Red 120 molecules could not be used for HSA attachment.

Effect of HSA concentration

Figure 4 shows the effects of concentration of HSA adsorbed. As seen in this figure, with an increase in HSA concentration in solution, the adsorbed amount of HSA per unit mass of beads increases until about

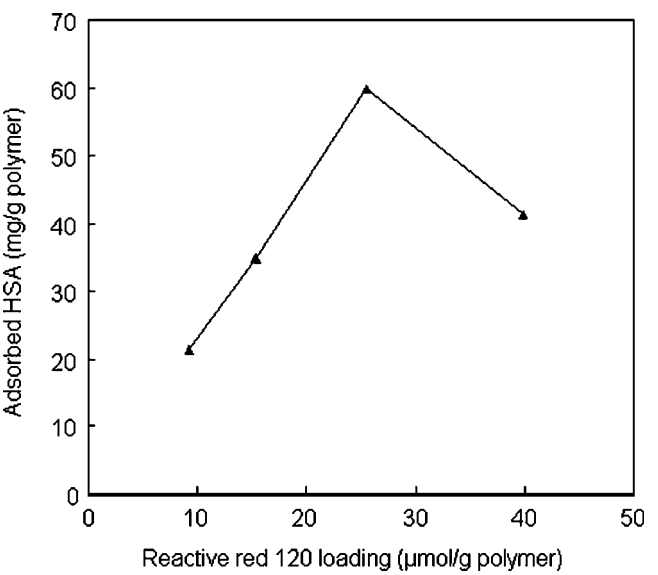


Figure 3 The adsorbed amount of HSA as a function of dye content; HSA concentration: 1.0 mg/mL; pH: 5.0; *T*: 25°C.

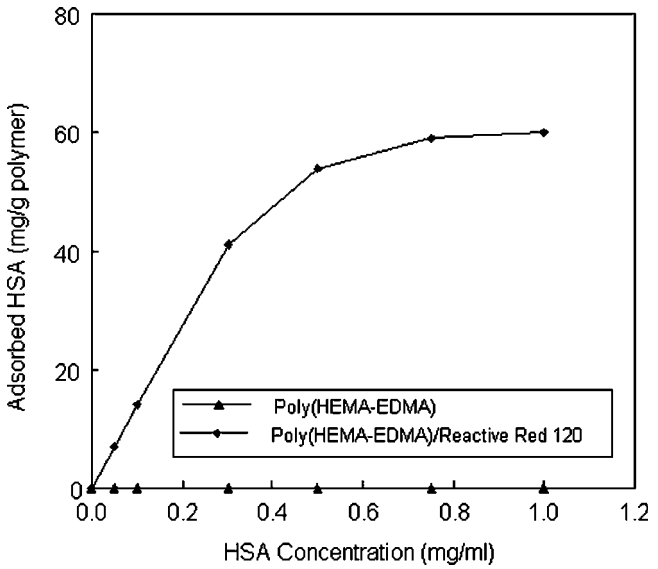


Figure 4 The adsorbed amount of HSA as a function of initial HSA concentration: Dye content: 25.5 μmol/g; pH: 5.0; *T*: 25°C.

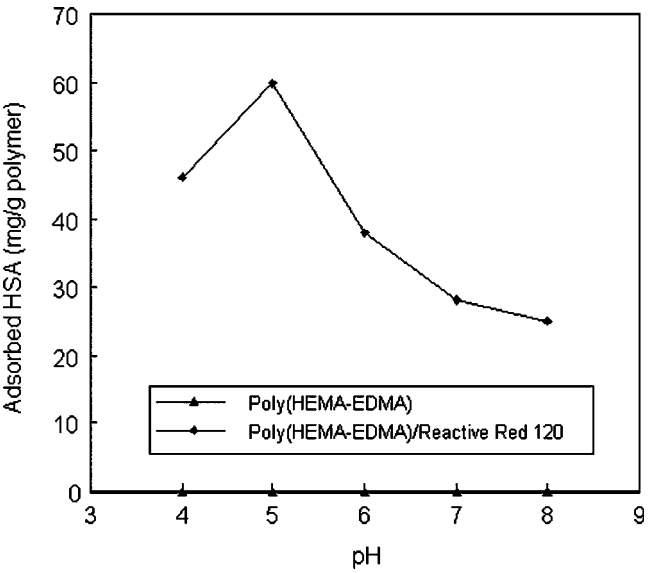


Figure 5 The adsorbed amount of HSA as a function of pH: Dye content: 25.5 μmol/g; HSA concentration: 1.0 mg/mL; *T*: 25°C.

0.5 mg/mL, and then approaches saturation. Nonspecific adsorption of HSA on the plain poly(HEMA–EGDMA) beads is 0.20 mg/g. Reactive Red 120 attachment increased the HSA adsorption amount of the beads (60 mg/g). It is clear that this increase in adsorption amount is because of specific interaction between attached dye molecules (i.e., Reactive Red 120) and HSA molecules which promote the adsorption of HSA.

A plot of linear Langmuir equation C_e/Q_e versus C_e is plot and the value of isotherm constants, equilibrium monolayer capacities (Q_e) and standard deviation of linear regression are given in Table I. The equilibrium monolayer adsorption amount, Qmax, is found to be 172 mg/g. The Langmuir adsorption equation provided an accurate description of the experimental data, which is confirmed by the extremely high values of the correlation coefficients for HSA. The R_L value given in Table I, shows that the adsorption behavior of HSA onto the Reactive Red 120 attached poly(HEMA–EGDMA) beads was favorable ($0 < R_L < 1$).

TABLE I
Langmuir Isotherm Constant

K_L	0.519
a_L	0.003
Q_{max}	172
R^2 (mg/g)	0.995
R_L	0.002
SD	0.224

Effect of pH

The amount of HSA adsorbed onto Reactive Red 120-attached poly(HEMA–EGDMA) beads as a function of the pH is shown in Figure 5. In all the investigated cases, the maximum adsorption of HSA is observed at pH 5.0, which is the isoelectric point of HSA. With the increase of pH above and below pH 5.0, HSA adsorption amount decreased. The decrease in the adsorption amount can be attributed to electrostatic repulsion effects between the identically charged groups. At the isoelectric points, proteins have no net charge and therefore, the maximum protein adsorption from aqueous solution in usually observed at these points.[19] In addition, these interactions between dye and protein molecules may result both from the ionization states of several groups on both the ligands (i.e., Reactive Red 120) and amino acid side chains in HSA structure, and from the conformational state of protein molecules at this pH. It should be also note that, nonspecific adsorption is independent of pH and it is observed to be the same at all the pH values studied.

Effect of ionic strength

The effect of ionic strength (adjusted by adding NaCl) on HSA adsorption is presented in Figure 6, which shows that the adsorption amount decreases with increasing ionic strength of the binding buffer (Acetate buffer, pH 5.0). The adsorption of the HSA decreases by about 82.0% as the NaCl concentration changes from 0.001 to 0.1*M*. The decrease in the

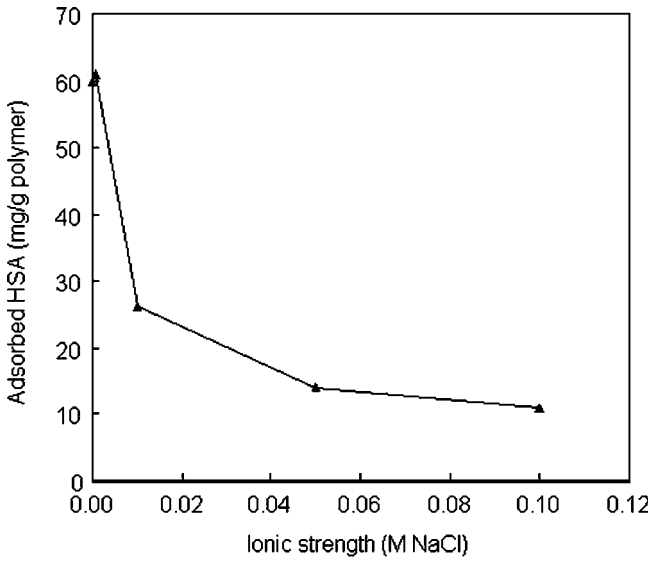


Figure 6 The adsorbed amount of HSA as a function of ionic strength: Dye content: 25.5 μmol/g; HSA concentration: 1.0 mg/mL; pH: 5.0; *T*: 25°C.

adsorption amount as the ionic strength increases can be attributed to the repulsive electrostatic interactions between the dye-attached beads and protein molecules. When the salt concentration increases in the adsorption medium, protein adsorption is low because of the coordination of the deprotonated sulfonate groups of the dye with sodium ions of salt. The distortion of existing salt bridges in the presence of salt also contributed to this low protein adsorption at high ionic strength.

Desorption studies

Desorption of the adsorbed HSA from the Reactive Red 120 attached poly(HEMA–EGDMA) beads is studied in a batch experimental setup. Beads carrying different amounts of HSA are placed within a desorption medium containing 0.1*M* Tris/HCl buffer containing 0.5*M* NaCl, and the amount of HSA released in 60 min is determined. More than 95.0% of the adsorbed HSA is removed in all cases when 0.1*M* Tris/HCl buffer is used for desorption. The addition of elution agent changed the charge of the peptide side groups because of their isoelectric points, resulting in the detachment of the HSA molecules from dye molecules. Note that, there is no Reactive Red 120 release in this case, which shows that dye molecules are attached strongly to poly (HEMA–EGDMA) beads. With the desorption data obtained, it can be concluded that Tris/HCl is a suitable desorption agent and allows repeated use of the affinity beads used in this study.

To show the reusability of the Reactive Red 120-attached poly(HEMA–EGDMA) beads, adsorption–desorption cycle of HSA are repeated five times with the same dye-affinity beads. As shown in Figure 7, the adsorption capacities for the polymeric beads did not noticeably change during the repeated adsorption–desorption operations. By taking into account the different experimental parameters studied above, it should be possible to scale-up the process of HSA separation by dye affinity chromatography on Reactive Red 120 attached poly(HEMA–EGDMA) beads.

HSA adsorption from human plasma

Table II shows the adsorption for human serum obtained from a healthy donor. There is a low adsorption of HSA (1.28 mg/g) on the poly(HEMA–EGDMA) beads, while much higher adsorption values (95.7 mg/g) are obtained when Reactive Red 120-attached poly(EGDMA-GMA) beads were used. The purity of HSA is assayed by SDS-PAGE. The purity of HSA was found to be 88%. It is worth to note that adsorption of HSA onto the Reactive Red 120-attached poly(HEMA–EGDMA) beads is ~1.6-fold higher than those obtained in the studies in which aqueous solutions are used. This may be explained as follows: the conformational structure of HSA molecule within their native environment (i.e., human plasma) is much more suitable for specific interaction with the Reactive Red 120-attached poly (HEMA–EGDMA) beads.

Competitive protein adsorption is also carried out and interesting results are obtained in these studies. Adsorption capacities were achieved as 2.0 mg/g for fibrinogen and 5.4 mg/g for γ-globulin. The total

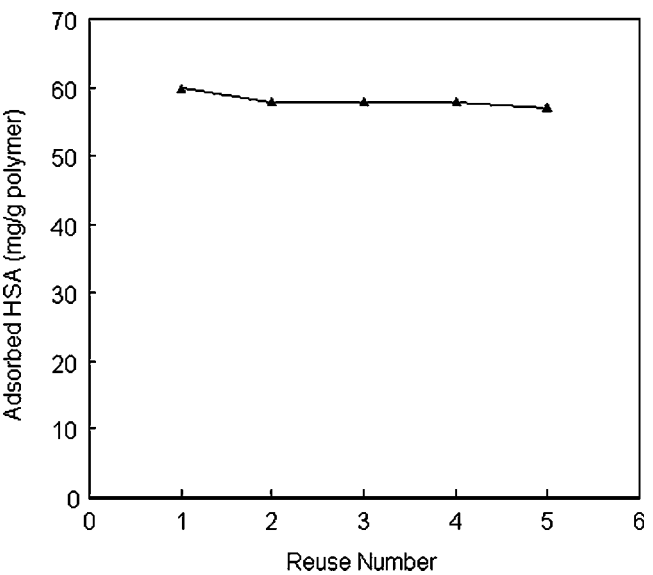


Figure 7 Repeated use of the Reactive Red 120-attached beads; Reactive Red 120 content: 25.5 μmol/g; HSA concentration: 1.0 mg/mL; pH: 5.0; *T*: 25°C.

TABLE II
HSA Adsorption from the Plasma of a Healthy Donor

Dilution agent	HSA concentration (mg/mL)	Adsorption amount (mg/g)
Plasma (undiluted)	37.7	95.7 ± 1.79
1/2 diluted plasma	18.9	52.5 ± 1.58
1/10 diluted plasma	9.4	36.4 ± 1.63

Reactive Red 120 content: 25.5 μmol/g; *T*: 25°C.

protein adsorption was determined as 103.8 mg/g. It is worth noting that adsorption of other plasma proteins (i.e., fibrinogen and γ-globulin) on the Reactive Red 120-attached poly(HEMA–EGDMA) beads are negligible. It should be noted that HSA is the most abundant protein in plasma. It generally makes up more than half of the total plasma proteins. It may be concluded that this low adsorption of fibrinogen and γ-globulin is because of the high concentration of HSA.

Comparison with related literature

Different dye-affinity adsorbents with different adsorption capacities were reported in literature for albumin adsorption. Ma et al. used Cibacron Blue F3GA-coupled nonporous magnetic poly(styrene-divinyl benzene) microspheres and they reported bovine serum albumin (BSA) adsorption capacities around 80.2 mg/g polymer.[19] Denizli and coworkers used dye affinity adsorbents including monosize poly(styrene–HEMA) and poly(glycidyl methacrylate) beads, polyamide hollow fiber and magnetic poly (hydroxyethyl methacrylate) beads, poly(EGDMA-glycidyl methacrylate) monolith, and they obtained 53–189 mg/g polymer for bovine and HSA.[20–26] Garipcan and coworkers prepared histidine and cysteine containing hydroxyethyl methacrylate based pseudospecific affinity beads and they obtained 8.8–22.8 albumin adsorption amount.[27,28] Nash and Chase used poly(vinyl alcohol) coated poly(styrene-divinyl benzene) beads carrying different dye ligands.[29] They presented adsorption capacities of 11.7–27 mg HSA/g. Horstmann et al. used Cibacron Blue F3GA-incorporated Sepharose CL-6B with different narrow-range mean particle diameters and they reported BSA adsorption capacities around 5.4–12 mg/g moist gel.[30] Zhang et al. reported 108.7 mg/g adsorption amount with Cibacron Blue F3GA-attached chitosan microspheres.[31] Li and Spencer used Cibacron Blue F3GA-attached poly(ethylene imine) coated titania and achieved 4.4 mg HSA/g.[32] Chase reached 14 mg BSA/g with Cibacron Blue F3GA-attached Sepharose CL-6B.[33] Tuncel et al. reported 60 mg BSA/g polymer with Cibacron Blue F3GA-attached poly(vinyl alcohol)-coated monodisperse polystyrene beads.[34] Muller-Schulte et al. used several carriers made of different polymers and Cibacron Blue F3GA as the dye-ligand.[35] Their albumin adsorption values were in the range of 0.19–0.81 mg HSA per milliliter sorbent. Adsorption capacities of commercially available agarose-Cibacron Blue F3GA adsorbents (BioRad, CA) were reported as about 11 mg albumin per milliliter sorbent.[36] Comparison of these results show that Reactive Red 120-attached poly(HEMA–EGDMA) beads exhibit higher HSA adsorption capacities.

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