Depletion of Albumin From Human Serum by Monosize Affinity Beads

Eş boyutlu manyetik affinite partiküller ile insan serumundan albumin uzaklaştırılması

Research Article

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ABSTRACT

The hydrophobic affinity ligand L-tryptophan immobilized magnetic poly(glycidyl methacrylate) [m-poly(GMA)] beads in monosize form (1.6μm in diameter) were used for the affinity depletion of human serum albumin [HSA]. The m-poly(GMA) beads were prepared by dispersion polymerization in the presence of Fe_3O_4 nano-powder. The epoxy groups of the m-poly(GMA) beads were converted into amino groups by using 1,6 diaminohexane (i.e., spacer arm). L-tryptophan was then covalently immobilized on spacer arm attached m-poly(GMA) beads. Elemental analysis of immobilised L-tryptophan for nitrogen was estimated as 42.5 μmol/g polymer. Adsorption studies were performed under different conditions in a batch system (i.e., medium pH, protein concentration and temperature). Maximum lysozyme adsorption amount of m-poly(GMA) and L-tryptophan immobilized m-poly(GMA) [m-poly(GMA)-L-tryptophan] beads were 1.78 and 172.9 mg/g, respectively. The applicability of two kinetic models including pseudo-first order and pseudo-second order model was estimated. It was also observed that after 5 adsorption–elution cycle, m-poly(GMA)-L-tryptophan beads can be used without significant loss in HSA adsorption capacity. The elution results demonstrated that the adsorption of HSA to the adsorbent was reversible.

Key Words

Magnetic Monosize Beads, Human Serum Albumin Depletion, Poly(glycidyl methacrylate).

ÖZET

L-Triptofan immobilize edilmiş manyetik poli(glisidil metakrilat) (1.6 μm çapında) partiküller [m-poly(GMA)] insan serum albumin [HSA] serumdan uzaklaştırılmasında kullanılmıştır. m-poly(GMA) partiküller Fe_3O_4 varlığında dispersiyon polimerizasyonu ile hazırlanmıştır. [m-poly(GMA)] partiküllerin epoksi grupları 1,6 diaminohekzan kullanılarak amino gruplarına çevrilmiştir. İmmobilize edilmiş L-triptofan miktarı elemental analiz ile 42.5 μmol/g polimer olarak hesaplanmıştır. Kesikli deney sistemi kullanılarak çeşitli koşullarda (örneğin ortam pH'ı, protein derişimi, sıcaklık) adsorpsiyon çalışmaları yapılmıştır. Maksimum HSA adsorpsiyonu m-poly(GMA) ve L-triptofan immobilize m-poly(GMA) [m-poly(GMA)-L-triptofan partiküller için 1.78 ve 172.9 mg/g bulunmuştur. Yalancı birinci ve ikinci derece kinetic modellemeler uygulanmıştır. Ayrıca 5 defa adsorpsiyon-desorpsiyon çalışmasında sonra [m-poly(GMA)-L-triptofan partiküllerin HSA adsorpsiyon kapasinde düşüş olmadığı görülmüştür. Elüsyon çalışmaları HSA'nın adsorbente bağlanmasının geri dönüşümlü olduğunu göstermiştir.

Anahtar Kelimeler

Mikron Boyutlu Manyetik Partiküller, İnsan Serum Albumini, Poli(glisidil metakrilat).

INTRODUCTION

Isolation, separation and purification of various types of proteins, peptides and other specific biomolecules are used in almost all branches of biosciences and biotechnologies. Separation science and technology is thus very important area which is necessary for further developments in research and technology. Separation of biomolecules is dependent on their biological and physico-chemical properties: molecular size, net charge, biospecific characteristics and hydrophobicity, respectively [1]. A wide variety of protein purification techniques are available today, however, different types of chromatography have become dominant due to their high resolving power such as dye-affinity chromatography, ion-exchange chromatography, immobilized metal-affinity chromatography, bioaffinity chromatography and hydrophobic interaction chromatography (HIC), [2], [3], [4] and [5].

HIC is based on hydrophobic interactions between immobilized hydrophobic ligands and non-polar regions on the surface of the proteins [6]. Hydrophobic groups on the protein bind to hydrophobic groups on the matrix. As the hydrophobicity of a protein increases, interaction between the ligand and the protein increases. The adsorption increases with high salt concentration in the mobile phase and the elution is achieved by decreasing the salt concentration of the eluent. HIC is also termed as salt-promoted [7]. HIC has been studied by many researchers and today it is a powerful bioseparation technique in laboratory-scale, as well as in industrial-scale purification of proteins [8]. Large variety of stationary phases was developed as stationary phase for HIC. And it is widely used in many applications for purification of biomolecules, such as n serum proteins, nuclear proteins, hormones, recombinant proteins and enzymes [9].

Non-polar amino acids such as alanine, methionine, tryptophan and phenylalanine on their surface can be used as a ligand in HIC [10], [11]. These pseudospecific ligands have high selectivity resulting from the cumulative effects of multiple weak binding forces such as; electrostatic, hydrophobic, hydrogen binding and van der Waals interactions.

Micron-sized magnetic beads are currently being used in large number of applications in many fields including biotechnology, biochemistry, colloid sciences and medicine [12] and [13]. 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 promise to solve many of the problems associated with chromatographic separations in packed bed and in conventional fluidized bed systems [14]. Magnetic separation is relatively rapid and easy, requiring a simple apparatus, composed of centrifugal separation. Recently, there has been increased interest in the use of magnetic carriers in protein purification [15].

Serum proteins may often serve as indicators of disease and is a rich source for biomarker discovery. However, the large number of proteins in serum makes the analysis very difficult because high abundant proteins tend to mask of those of lower abundance [16]. The high abundance of HSA and immunoglobulin which comprise about 80% of total serum protein, is a major problem in proteome studies. Depletion of abundant serum proteins will help in the discovery and detection of less abundant proteins that may prove to be informative disease markers [17]. A variety of depletion methods for specific removal of high abundant proteins from body fluids have been developed. Several major strategies are available concerning the mechanims of removal of HSA and IgG. In the case of HSA, depletion can be achieved by either dye-ligands such as the widely recognized Cibacron Blue F3GA and derivatives thereof [18], or specific antibodies [19]. A dye affinity resin for removal of HSA has the advantage of high loading capacity as compared to an antibody based system but has been shown to lack of specificity [20, 21]. Other methods reported include a proprietary polypeptide affinity matrix that removes HSA together with IgG, but is now apparently unavailable [22], and a method based on the size separation in a centrifugal filtration device that was, perhaps predictably, unsuccessful [23].

In our previous works, poly(glycidyl methacrylate) beads [poly(GMA)] were prepared in monosize form by dispersion polymerization.

The Cibacron Blue F3GA-modifed non-magnetic poly(GMA) beads were used in affinity depletion of HSA from human serum for proteome studies [24], IgG depletion from human serum [25], and recombinant interferon-α [26], respectively. We also prepared magnetic poly(GMA) beads for lysozyme purification from egg white [5] and [27]. The goal of this study is to prepare a tryptophan containing monosize magnetic poly(glycidyl methacrylate) beads for efficient separation of HSA from human serum. The monosize m-poly(GMA) beads were obtained by dispersion polymerization of GMA. HSA adsorption properties of the pseudo-affinity beads from aqueous solutions were investigated at different experimental conditions in a batch system. Elution of HSA and reusability of the adsorbents were also tested.

EXPERIMENTAL

Materials

Human serum albumin (HSA, 98% pure by gel electrophoresis, fatty acid free, 67 kDa) was purchased from Aldrich Chem. Co., (Milwaukee, WI, USA) and used as received. Glycidyl methacrylate (GMA, Fluka A.G., Buchs, Switzerland) was purified by vacuum distillation and stored in a refrigerator until use. L-tryptophan was obtained from Sigma. Azobisisobutyronitrile (AIBN) was selected as the initiator. AIBN was recrystallized from methanol. Ethanol (Merck, Germany) was used as the diluent without further purification. All other chemicals were analytical grade reagents commercially available and used without further purification. Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust-free environment. 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 m-poly(GMA) beads

m-poly(GMA) monosize beads were synthesized as previously described elsewhere [27]. The dispersion polymerization was performed in a sealed polymerization reactor (volume: 500mL) equipped with a temperature control system. A typical procedure applied for the dispersion polymerization of GMA is given below. The monomer phase was comprised of 40mL GMA, 250mg AIBN and 1 g magnetite (Fe_3O_4) particles. The resulting medium was sonicated for about 5min at 200W within an ultrasonic water bath (Bransonic 2200, England) for the complete dissolution of AIBN in the polymerization medium. 4.0 grams of poly(vinyl pyrrolidone) was dissolved in 50% v/v aqueous ethanol solution and placed in a polymerization reactor. The reactor content was stirred at 500rpm during the monomer addition completed within about 5min and the heating was started. The initial-polymerization time was defined when the reactor temperature was raised to 65°C. The polymerization was allowed to proceed under nitrogen atmosphere at 70°C for 4h (stirring rate: 500rpm). After completion of the polymerization period, the reactor content was cooled down to room temperature and centrifuged at 5000rpm for 10min for the removal of dispersion medium. This polymerization reaction led to the formation of pale brown beads. m-poly(GMA) beads were redispersed within 10mL of ethanol and centrifuged again under similar conditions. The ethanol washing was repeated three times for complete removal of unconverted monomers and other components. Finally, m-poly(GMA) beads were redispersed within 10mL of water (0.10%, by weight) and stored at room temperature.

L-tryptophan immobilization

In order to prepare the L-tryptophan immobilized m-poly(GMA) beads, following procedure was applied. The epoxy groups of the m-poly(GMA) beads were converted into amino groups with 0.5M 1,6 diaminohexane (i.e., spacer arm). The m-poly(GMA) beads were incubated with 1,6 diaminohexane solution at 65 °C in a reactor containing 20 g of dry beads and shaked for 6 h. After the reaction, the spacer arm attached m-poly(GMA) beads were washed with distilled water. The spacer arm attached m-poly(GMA) beads were equilibrated in phosphate buffer (100 mL, 10 mM, pH 7.0) for 2 h and transferred to the activation solution containing glutaric dialdehyde (50 mL, 0.25%, v/v). The activation reaction was carried out at 25 °C for 12 h. After the reaction period, the excess glutaric dialdehyde was removed by washing sequantially the beads with distilled water and phosphate buffer. The activated beads were incubated in

phosphate buffer (10 mM, pH 7.0) for 4 h, They were transferred to the L-tryptophan solution mixture (3.0 g L-tryptophan/25mL phosphate buffer). This immobilization reaction was carried out under a constant gentle shaking (250 rpm) at 25 °C for 6 h. At the end of this reaction period, the L-tryptophan immobilized adsorbents were removed by filtration and washed extensively with methanol and water in order to remove weakly adsorbed L-tryptophan molecules and then dried in vacuum for 24 h. When not in use, the resulting adsorbents were kept under refrigeration in 0.02% NaN_3 solution for preventing of microbial contamination.

Characterization of monosize beads

The amounts of 1,6 diaminohexane and L-tryptophan immobilized on the m-poly(GMA) beads were determined by measuring the nitrogen content in the polymer structure with elemental analysis (LECO, CHNS-932, USA). FTIR spectra of the m-poly(GMA) beads, 1,6 diaminohexane modified m-poly(GMA) beads and m-poly(GMA)-L-tryptophan 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), and pressed into a tablet, and the spectrum was then recorded.

In order to estimate the amount of leached magnetite from the m-poly(GMA) beads, the beads (250 mg) were placed in test tube containing 10 mL of leach media and shaken on a rotary shaker for 24 h. The amount of magnetite leached into the medium was determined by a graphite furnace atomic absorption spectrophotometer (AAS 5EA, Carl Zeiss Technology, Zeiss Analytical Systems, Germany). Three kinds of release media were used: 50% acetic acid solution (pH 2.0), 50 mM phosphate buffer solution (pH 7.0) and 50 mM sodium citrate/NaOH buffer solution (pH: 12.0).

The leakage of the L-tryptophan from the adsorbents was followed by incubating the 250 mg fully wetted adsorbents with 10 mL of phosphate buffered saline (PBS, pH 7.4) solution for 24 h at room temperature. The leakage experiments were carried out at 25 °C at a stirring rate of 50 rpm. L-tryptophan released after this incubation was measured in the liquid phase spectrophotometrically.

### HSA adsorption-desorption studies from aqueous solutions

Adsorption of HSA on the m-poly(GMA)-L-tryptophan beads from aqueous solutions was investigated batch-wise. The beads (0.05g) were incubated with 10mL of the aqueous solutions of HSA for 2h (i.e. equilibrium time), in flasks stirred at 150rpm. Effects of the HSA concentration, pH of the medium, and temperature on the adsorption capacity were studied. 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 the initial concentration of HSA on adsorption, it was changed between 0.5 and 3.0mg/mL. To observe the effects of the temperature on the adsorption, adsorption studies were carried out between 4 and 37°C. Samples were withdrawn at suitable time intervals and HSA concentration was determined by measuring the initial and final concentration of HSA within the adsorption medium using Coomassie Brilliant Blue as described by Bradford [28]. The amount of adsorbed HSA was calculated using mass balance. Each experiment was performed in twice for quality control and statistical purposes.

Desorption and repeated use

The desorption of the adsorbed HSA from the m-poly(GMA)-L-tryptophan beads was studied in batch experimental setup. HSA adsorbed m-poly(GMA) beads were placed in desorption medium, containing 0.1M ethylene glycol solution and stirred continuously (at stringing rate 150rpm) for 1h at room temperature. The final HSA concentration in the desorption medium was determined by Bradford method [28]. In order to show the reusability of the beads, adsorption-desorption cycle of HSA was repeated five times by using the same beads.

RESULTS AND DISCUSSION

Incorporation of amino acids into polymer matrix has been gaining much attention in affinity separation. Aminoacids that are used as pseudospecific ligands have certain advantages. They are more stable than bioligands because they do not require a specific tertiary structure for maintaining their activities. Leakage of aminoacids into the medium does not occur, since they are incorporated into the polymer structure.
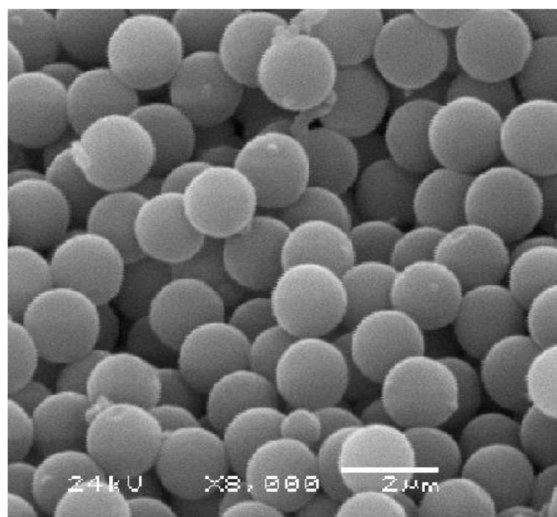
Figure 1. SEM photograph of monosize m-poly (GMA) beads.

Aminoacids are very reactive with different chemical substances. The higher flexibility and durability of these ligands as well as significantly lower material and manufacturing costs are also very important [10].

In this study, a novel trptophan immobilized monosize m-poly(GMA) beads were prepared successfully as affinity sorbents for efficient separation of HSA from aqueous solutions. L-tryptophan was immobilized on m-poly(GMA) beads. Effect of parameters (pH, HSA concentration, and temperature) on HSA adsorption capacity of m-poly(GMA)-L-tryptophan beads was investigated.

Characteristics of monosize m-poly(GMA) beads

Monosize m-poly(GMA) beads (RSD<1%, 1.62μm in diameter) were obtained by dispersion polymerization. The physicochemical properties of beads are presented in Table 1. The morphology and structure of the resulting beads were observed by SEM picture as shown in Figure 1. As seen here, the m-poly(GMA) beads were highly uniform in size. Polydispersity index (PDI) value of m-poly(GMA) beads was calculated to be around 1.008.

The theoretical content of epoxy groups, calculated on the basis of the feed composition, i.e. GMA content in the monomer mixture was 4.2 mmol/g. The content of epoxy groups on the surface of the poly(GMA) sample determined by the HCl-pyridine method differs from the theoretical value

Table 1. Some properties of the monosize m-poly(GMA) beads.

Particle diameter	1.62 ± 0.01 μm
Polydispersity index	1.008
Specific surface area	3.2 m²/g
Theoretical epoxy group content	4.2 mmol/g
Experimental epoxy group content	3.38 mmol/g
Swelling ratio	45 %
Wet density	1.09 g/mL
Fe_3O_4 incorporation	4.5%
Resonance of magnetic field	2055 Gauss
g factor	2.28
1,6-Diaminohexane content	80.8 μmol/g
L-tryptophan attachment	42.5 μmol/g

(3.8 mmol/g). Some of the epoxy groups usually remain inside a poly(GMA) beads and are not accessible for subsequent reactions or for analytical determinations.

Pseudospecific ligand L-tryptophan was joined onto m-poly(GMA) polymeric structure. The studies of L-tryptophan and magnetite leakage from the m-poly(GMA)-L-tryptophan beads showed that there was no L-tryptophan and magnetite leakage in any medium used throughout this study, even in long storage period of time (more than 20 weeks).

FTIR spectra of plain and modified m-poly(GMA) beads was shown in Figure 2. The FTIR spectrum of m-poly(GMA) has the characteristic stretching vibration band of hydrogen-bonded alcohol at 3500cm⁻¹. Among the characteristic vibrations of GMA is the methylene vibration at 2930cm⁻¹. The epoxide group gives the band at 850 and 910cm⁻¹ (epoxy ring). The vibration at 1740cm⁻¹ represents the ester configuration of GMA. In Figure 2(B), the FTIR spectrum of 1,6 diaminohexane modified m-poly(GMA) beads has the characteristic N–H amine stretching bands at between 3500 and 3300 cm⁻¹ (the bands more broadened after attachment of 1,6 diaminohexane). The most important absorption band at 1650 cm⁻¹ repsenting N–H bending, is due to 1,6 diaminohexane attachment to the m-poly(GMA) beads. After glutaric dialdehyde activation, in Figure 2 (C), the intensity of the N–H band decreased due to the reaction took place between amine and aldehyde groups. In this spectrum, amine peak was decreased, while the C=O band at 1740 cm⁻¹ as broadened compared to inactivated beads due to reaction with amine groups to yield the C=O bands. The absorption bands of the functional
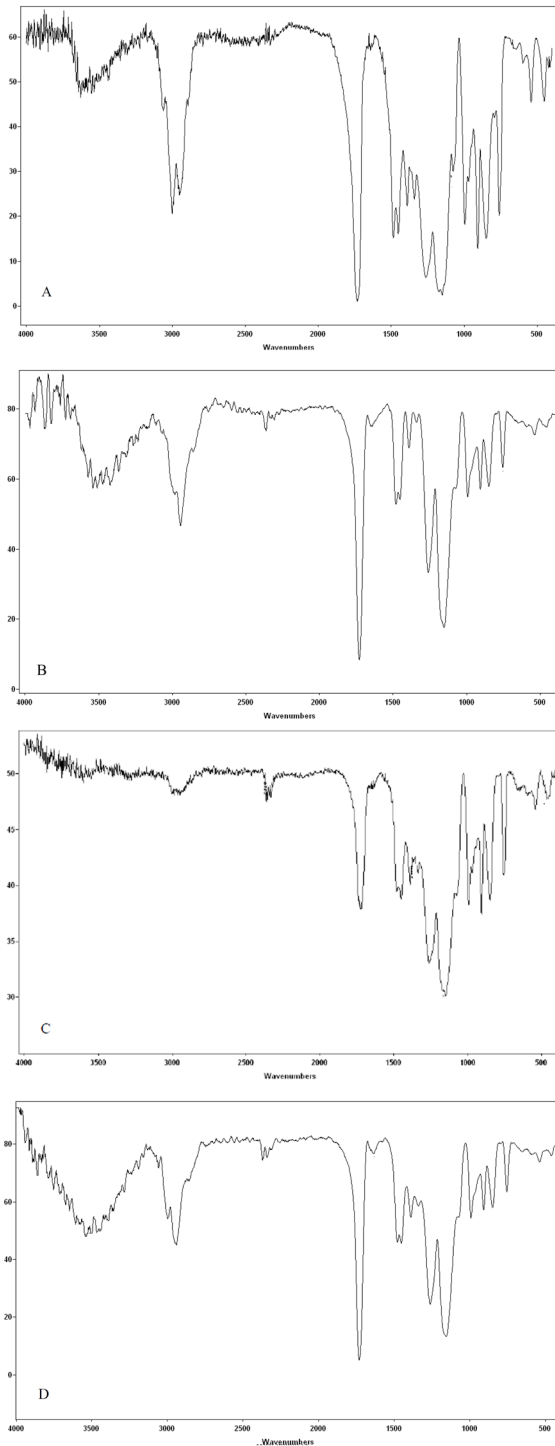
Figure 2. FTIR spectra of: (A) m-poly(GMA); (B) 1,6 diaminohexane modified m-poly(GMA) beads; (C) glutaric dialdehyde activated m-poly(GMA) beads; (D) m-poly(GMA)-L-tryptophan.

groups of the L-tryptophan can be clearly seen in Figure 2 (D), 3400 cm⁻¹ and 3100 cm⁻¹ absorption bands are due to N-H stretching and aromatic C-H stretching, respectively. The intensive peak 1370 cm⁻¹ corresponds to aromatic C-N stretching.

Adsorption of HSA from aqueous solutions
Effect of pH
Figure 3. shows HSA adsorption capacity at different pH values. At pH values lower and higher than pH 5.0, the adsorbed amount of HSA drastically decreased. The maximum adsorption capacity (100.7 mg/g polymer) was observed at pH 5.0 indicating that the binding of HSA on the m-poly(GMA)-L-tryptophan beads should involve hydrophobic interactions with a high percentage according to the other interactions.

Significantly lower adsorption capacities were obtained in more acidic and in more alkaline pH regions. The decrease in the HSA adsorption capacity in more acidic and more alkaline pH regions can be attributed to electrostatic repulsion effects between the identical charged protein molecules on the surface of the m-poly(GMA)-L-tryptophan beads. As it has been shown that proteins have no net charge at their isoelectric points and so the protein solubility in auqeous media decreases. Thus the maximum adsorption from aqueous solutions is usually observed at their isolectric points [24]. The isoelectric pH of HSA is 4.9 [29]. However, acidic and basic medium caused the protein to be positively or negatively charged, increasing the solubility of protein in media.
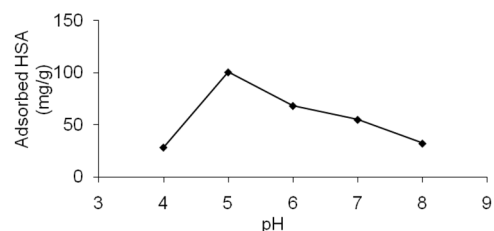
Figure 3. Effect of pH on HSA adsorption; HSA concentration: 1.0 mg/mL; incubation time: 2 h; temperature: 25°C.

Effect of HSA concentration
Figure 4. shows the HSA adsorption isotherm. A point worth noting that, there was a low non-specific HSA adsorption (i.e., the adsorption onto the plain m-poly(GMA) beads about 1.78 mg/g. There are no reactive binding groups or sites onto m-poly(GMA) which interact with HSA molecules. Hence, this non-specific adsorption may be due to weak interactions (van Der Waals interaction and hydrogen binding) between HSA and epoxy groups on the surface of m-poly(GMA) beads. L-tryptophan attachment

Table 2. Equilibrium adsorption constants.

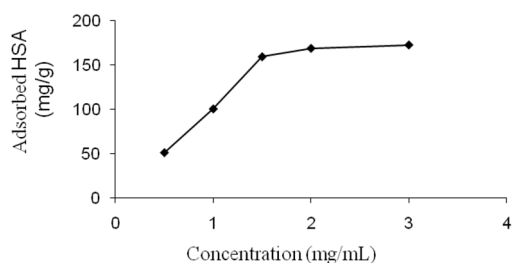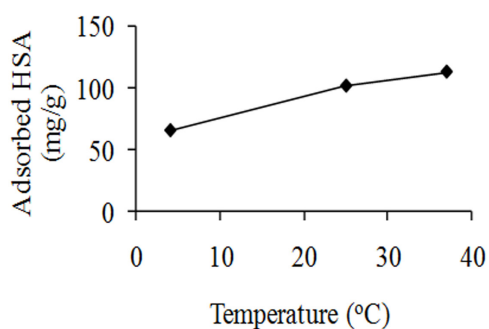| Langmuir model			Freundlich model		
Q_{max} (mg/g)	b(mL/g)	R^2	K_F	1/n	R^2
208.3	3.4	0.951	178.9	0.496	0.739

significantly increased the HSA adsorption capacity of the monosize beads (up to 172.9mg/g). The amount of HSA adsorbed per unit mass of the m-poly(GMA)-L-tryptophan beads increased first with the initial concentration of HSA then reached a plateau value which represents saturation of the active adsorption sites (which are available and accessible for HSA) on the monosize beads. This increase in the HSA adsorption capacity may have resulted from hydrophobic interactions caused by the L-tryptophan and by hydrophobic amino acids on the HSA molecules. It should be mentioned that L-tryptophan is hydrophobic overall, and it prefers to interact with hydrophobic groups in HSA structure.

Effect of temperature

Effect of temperature on the adsorption of HSA onto m-poly(GMA)-L-tryptophan beads was presented in Figure 5. Adsorption of HSA on the beads was significantly increased with increasing temperature indicating that hydrophobic interactions were much more significant in the adsorption. As known, in HIC, increasing the temperature enhances protein retention and lowering the temperature generally promotes the protein elution [30]. In fact, the HIC, is an entropy-driven process [$\Delta G=(\Delta H-T\Delta S) \sim -T\Delta S$]. Since ΔH may be a small positive or negative value, ΔG is controlled by a positive entropy change and thus increases with temperature. The calculated ΔH value of the system for the HSA interaction with the m-poly(GMA)-L-tryptophan beads was -5.83kcal/mol. It is interesting to note that the van der Waals attraction forces, which operate in hydrophobic interactions, also increase with increasing temperature.

Figure 4. Adsorption isotherms of HSA; pH 5.0; incubation time 2h; temperature 25 °C.

Figure 5. Effect of temperature on HSA adsorption. HSA concentration: 1.0mg/mL; pH 5.0; incubation time 2h.

Adsorption isotherms

Two important physico-chemical aspects for evaluation of the adsorption process as a unit operation are the kinetics and the equilibria of adsorption. Modelling of the equilibrium data has been done using the Langmuir and Freundlich isotherms. The Langmuir and Freundlich isotherms are represented as follows Eqs.(1) and (2), respectively.

$$Q = Q_{max}. b. C_{eq} / (1 + bC_{eq}) \qquad (1)$$

$$Q_{eq} = K_F (C_{eq})^n \qquad (2)$$

Table 3. The first- and second-order kinetic constants.

C_{in}	Experimental	1st Order			2nd Order		
	Q_{eq}	Q_{eq}	k_1	R^2	Q_{eq}	k_2	R^2
0.5	51.3	63.5	0.03455	0.928	90.9	0.000138	0.833
1.0	100.7	124.2	0.02994	0.917	263.2	0.000014	0.533
1.5	159.6	188.4	0.02533	0.909	333.3	0.000009	0.549
2.0	169.1	200.0	0.02533	0.907	384.6	0.000010	0.575
3.0	172.9	207.0	0.02994	0.936	476.2	0.000012	0.737

Table 4. Elution of HSA.

C_{in} (mg/mL)	HSA adsorbed (mg/g)	HSA eluted (%)
0.5	51.3	83.2
1.0	100.7	85.1
1.5	159.6	85.5
2.0	169.1	86.7
3.0	172.9	88.4

where b is the Langmuir isotherm constant, K_F is the Freundlich constant, and n is the Freundlich exponent. $1/n$ is a measure of the surface heterogeneity ranging between 0 and 1, becoming more heterogeneous as its value gets closer to zero. The value of Q_{eq} gives the theoretical monolayer saturation capacity of monosize beads. Some model parameters were determined by nonlinear regression with commercially available software and are shown in Table 2. Comparison of all theoretical approaches used in this study shows that the Langmuir equation fits the experimental data best.

Adsorption kinetics modeling

Kinetic model is used to examine the controlling mechanism of adsorption process such as mass transfer and chemical reaction. Kinetic models are used to test experimental data. Pseudo-first- and second-order equations are the kinetic models, and can be used to correlate that measured concentrations are equal to adsorbent surface concentrations. Lagergren first-order rate equation may be represented as follows:

$$\Delta Q_t / dt = k_1 (Q_{eq} - Qq_t) \qquad (3)$$

where k_1 is the rate constant of pseudo-first order adsorption (min^{-1}) and q_{eq} and q_t (mg/g) denote the amounts of adsorbed protein at equilibrium and at time t (min), respectively. After integration by applying boundary conditions, $Q_t=0$ at $t=0$ and $Q_t=q_t$ at t=t, gives;

$$\log [Q_{eq}/(Q_{eq}-Q_t)] = (k_1t)/2.303 \qquad (4)$$

Eq. (4) can be rearranged to obtain a linear form;

$$\log(Q_{eq}-Q_t) = \log(Q_{eq}) - (k_1t)/2.303 \qquad (5)$$

when $\log(Q_{eq})$ versus t is plotted, line must be straight to confirm the applicability of the kinetic model. In a true first-order process $\log(Q_{eq})$ should be equal to the interception point of a plot of $\log(Q_{eq}-Q_t)$ versus t.

In addition, a pseudo-second order equation based on adsorption equilibrium capacity may be expressed in the form;

$$\Delta Q_t / dt = k_2 (Q_{eq} - Q_t)^2 \qquad (6)$$

Where; k_2 (g mg^{-1} min^{-1}) is the rate constant of pseudo-second order adsorption process. Integrating Eq. (6) and applying the boundary conditions, $q_t=0$ at $t=0$, and $q_t=qt$ at t=t, leads to,

$$[1/(Q_{eq}-Q_t)] = (1/Q_{eq}) + k_2t \qquad (7)$$

or equivalently for linear form

$$(t/Q_t) = (1/k2Qeq^2) + (1/Q_{eq}) t \qquad (8)$$

a plot of t/q_t versus t gives a linear relationship in the second-order kinetics. The rate constant (k_2) and adsorption at equilibrium (Q_{eq}) can be obtained from the intercept and slope, respectively.

The comparison of experimental adsorption capacity and the theoretical value estimated from the previous equations, are presented in Table 3. The theoretical Q_{eq} value estimated from the second order kinetic model gave significantly different value compared to experimental value, and the correlation coefficient was also found to be lower. These results showed that the second order kinetic model is improper for these affinity beads.

Desorption and reusability of adsorbents

Desorption of HSA was studied with 0.1M ethylene glycol solution in a batch system. HSA adsorbed beads were placed within the desorption medium and stirred continuously at 150rpm for 1h at room temperature. The final HSA concentration in the desorption media was determined by Bradford Protein Assay at 595nm. Table 4 gives the elution data. More than 83% of the adsorbed HSA was eluted in all cases when ethylene glycol was used for elution.

In order to test the reusability of the beads, HSA adsorption–desorption procedure was repeated five times by using the same beads. The non-polar parts of ethylene glycol compete effectively with the bound proteins for the adsorption sites on of beads, resulting in the displacement of the latter.
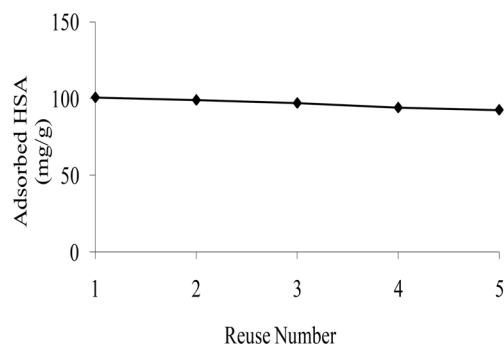
Figure 6. Reusability of beads; HSA concentration: 1.0mg/mL; pH 5.0; incubation time 2h; temperature 25°C.

Also, it decreases the surface tension of water thus weakening the hydrophobic interactions to give a subsequent dissociation of the ligand-solute complex. At the end of five adsorption–desorption cycle, there was no remarkable reduction in the adsorption capacity as seen in Figure 6.

CONCLUSION

Chromatographic techniques for protein purification have a number of drawbacks, such as the compressibility of the column packaging materials (i.e., softgel and macrobeads) and the fouling. Commercially available polymer based porous adsorbents exhibit surface areas 200–500m^2 or even larger per unit mass of the adsorbent. However, these type of adsorbents have also important disadvantages. First of all the adsorption rates are much slower, because of mainly the pore diffusion resistance. In addition, the high active surface area of these sorbents is mainly due to the fine pores in the matrix, which are not available for large solute molecules. In other words, large molecules cannot penetrate within these pores and therefore cannot use the active surface area available, which means low adsorption capacities for large molecules. In order to increase the protein loading capacity, the particle size has been reduced to 0.1–1.0μm, but such carriers require high pressure equipments. Due to these reasons, non-porous affinity sorbents of small particle diameter (i.e., micron size) have been gaining more attention since the mid-1980s for the rapid high-performance liquid chromatography (HPLC) of biomolecules. It appears that the monosize m-poly(GMA)-L-tryptophan beads can be applied for the purification of proteins. Our goal was to find a cost effective and reusable hydrophobic affinity beads having high adsorption capacity for purification of HSA from human serum. Based on our evaluations of adsorption capacity, recovery and binding specificity of the hydrophobic-affinity beads offered the promising purification approach.

REFERENCES

1.   J.A. Queiroz, F.A.P. Garcia, J.M.S.Cabral, Hydrophobic interaction chromatography of Chromobacterium viscosum lipase on polyethylene glycol immobilized on Sepharose, J. Chromatogr. A, 734 (1996) 213.

2.   F.A.P. Garcia, E.M.V. Pires, Chromatography, in: J.F. Kennedy, J.M.S. Cabral, (Eds.), Wiley, London, pp., (1993), 415.

3.   E.B. Altıntaş, A. Denizli, Monosize poly(glycidyl methacrylate) beads for dye-affinity purification of lysozyme, Int. J. Biol. Macromol., 38 (2006) 99.

4.   E.B. Altintas, N. Tüzmen, L. Uzun, A. Denizli, Immobilized metal affinity adsorption for antibody depletion from human serum with monosize beads, Ind., Eng. Chem. Res. 46 (2007) 7802.

5.   E.B. Altıntaş¸ A. Denizli, Monosize magnetic hydrophobic beads for lysozyme purification under magnetic field, J. Mat. Sci. Eng. C, 29 (2009) 1627.

6.   J.L. Fausnaugh, F.E. Regnier, Solute and mobile phase contributions to retention in hydrophobic interaction chromatography of proteins, J. Chromatogr., 359 (1986) 131.

7.   J. Porath, Salt-promoted adsorption: recent developments, J. Chromatogr. 376 (1986) 331.

8.   E. Boschetti, Antibody separation by hydrophobic charge induction chromatography, Trends Biotechnol. 20 (2002) 333.

9.   S. Oscarson, J. Porath, Protein chromatography with pyridine- and alkylthioether-based agarose adsorbents, J. Chromatogr. 499 (1990) 235.

10.   O. Pitiot, C. Legallais, L. Darnige, M.A. Vijayalakshmi, A potential set up based on histidine hollow fiber membranes for the extracorporeal removal of human antibodies, J. Membr. Sci. 166 (2000) 221.

11.   Ş. Öncel, L. Uzun, B. Garipcan, A. Denizli, Synthesis of phenylalanine-containing hydrophobic beads for lysozyme adsorption, Ind. Eng. Chem. Res., 44 (2005) 7049.

12.   Z.Y. Ma, Y.P. Guan, X.Q. Liu, H.Z. Liu, Synthesis of magnetic chelator for high-capacity immobilized metal affinity adsorption of protein by cerium initiated graft polymerization, Langmuir 21 (2005) 6987.

13. A. Denizli, H. Yavuz, B. Garipcan, M.Y. Arıca, Nonporous monosize polymeric sorbents: Dye and metal chelate affinity separation of lysozyme, J. Appl. Polym. Sci.76 (2000) 115.

14. M. Takafuji, S. Ide, H. Ihara, Z. Xu, Preparation of poly(1-vinylimidazole)-grafted magnetic nanoparticles and their application for removal of metal ions, Chem. Mater. 16 (2004) 1977.

15. Z.Y. Ma, Y.P. Guan, H.Z. Liu, Synthesis of monodisperse nonporous crosslinked poly(glycidyl methacrylate) particles with metal affinity ligands for protein adsorption, Polym. Int. 54 (2005) 1502.

16. K. Björhall, T. Miliotis, P. Davidsson, Comparison of different depletion strategies for improved resolution in proteomic analysis of human serum samples, Proteomics 5 (2005) 307.

17. L.F. Steel, M.G. Trotter, P.B. Nakajima, T.S. Mattu, G. Gonye, T. Block, Efficient and specific removal of albumin from human serum samples, Mol. Cell Proteomics 2 (2003) 262.

18. E. Gianazza, P. Arnaud, Chromatography of plasma proteins on immobilized Cibacron Blue F3-GA, J. Biochem. 203 (1982) 637.

19. Y.Y. Wang, P. Cheng, D.W. Chan, A simple affinity spin tube filter method for removing high-abundant common proteins or enriching low-abundant biomarkers for serum proteomic analysis, Proteomics 3 (2003) 243.

20. A. Denizli, E. Piskin, Dye-ligand affinity systems, J. Biochem. Biophys. Methods 49 (2001) 391.

21. N. Ahmed, G. Barker, K. Oliva, D. Garfin, An approach to remove albumin for the proteomic analysis of low abundance biomarkers in human serum, Proteomics 3 (2003) 1980.

22. B.A. Lollo, S. Harvey, J. Liao, A.C. Stevens, R. Wagenknecht, R. Sayen, J. Whaley, F.G. Sajjadi, Improved two-dimensional gel electrophoresis representation of serum proteins by using ProtoClear, Electrophoresis 20 (1999) 854.

23. H.M. Georgiou, G.E. Rice, M.S. Baker, Proteomic analysis of human plasma: Failure of centrifugal ultrafiltration to remove albumin and other high molecular weight proteins, Proteomics 1 (2001) 1503.

24. E.B. Altıntaş, A. Denizli, Efficient removal of albumin from human serum by monosize dye-affinity beads, J. Chromatogr. B., 832 (2006) 216.

25. E.B. Altıntaş, N. Tuzmen, N. Candan, A. Denizli, Use of magnetic poly(glycidyl methacrylate) monosize beads for the purification of lysozyme in batch system, J. Chromatogr., 853 (2007) 105.

26. E.B. Altıntaş, A. Denizli, Affinity adsorption of recombinant human interferon-α on monosize dye-affinity beads, J. Appl. Polym. Sci.103 (2007) 975.

27. E.B. Altıntaş, L. Uzun, A. Denizli, Synthesis and characterization of monosize magnetic poly(glycidyl methacrylate) beads, China Particulogy, 5 (2007) 174.

28. M.M. Bradford, A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding, Anal. Biochem., 1976, 72, 248.

29. C.A. Burtis, E.R. Ashwood, Tietz Fundamentals of Clinical Chemistry, 5th ed., Elsevier, New York, 2001.

30. Y.S. Ho, G. McKay, Pseudo-second Order Model for Sorption Processes, Proc. Biochem. 34 (1999) 451.