Adsorption of BSA on Metal Loaded Monosize p(GMA) Microspheres

Metal Yüklenmiş Eşboyutlu p(GMA) mikroküreleri BSA Adsorpsiyonu

Research Article

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ABSTRACT

Immobilized metal ion affinity chromatography (IMAC) is an efficient method for the adsorption of histidine-having proteins so poly(glycidyl methacrylate) [p(GMA)] monosize microspheres with an average diameter of 1.6 μm were produced by dispersion polymerization for adsorption of bovine serum albumin. Cu(II) ions were chelated on the poly(glycidyl methacrylate)-iminodiacetic acid [p(GMA)-IDA] microspheres to be used in bovine serum albumin adsorption studies in batch system. Characterizations of the microspheres were carried out by scanning electron microscopy (SEM), elemental analysis, Fourier transform infrared (FTIR) spectroscopy. The maximum adsorption capacity of the p(GMA)-IDA-Cu(II) microspheres was found to be 278.6 mg/g polymer at pH 5.0. The elution studies were performed by 1.0 M NaCl solution. The obtained results showed that the metal chelated p(GMA)-IDA-Cu(II) microspheres can be evaluated as an efficicent adsorbent for albumin adsorption.

Key Words
Immobilized metal ion affinity chromatography, Albumin, Proteomics, Poly(glycidyl methacrylate).

ÖZET

İmmobilize metal iyon afinite kromatografi (İMAK), histidin taşıyan proteinlerin adsorpsiyonu için etkili bir yöntem olduğundan sığır serum albuminin uzaklaştırılması için dispersiyon polimerizasyon yöntemiyle, 1.6 μm çapında eş boyutlu poli(glisidil metakrilat) [p(GMA)] mikroküreler üretilmiştir. Cu(II) iyonları, kesikli sistemde sığır serum albuminin adsorpsiyon çalışmalarında kullanılmak üzere p(GMA)-IDA mikroküreleri ile şelatlanmıştır. Mikrokürelerin karakterizasyonu taramalı elektron mikroskobu (SEM), elementel analiz, Fourier dönüşüm kızılötesi (FTIR) spektroskopisi kullanılarak gerçekleştirilmiştir. p(GMA)-IDA-Cu(II) mikrokürelerinin maksimum adsorpsiyon kapasitesi pH 5.0'te 278.6 mg/g polimer bulunmuştur. Elüsyon çalışmaları 1.0 M NaCl çözeltisi ile gerçekleştirilmiştir. Elde edilen sonuçlar p(GMA)-IDA-Cu(II) mikrokürelerin albüminin adsorpsiyonu için etkin bir adsorban olarak kabul edilebileceğini göstermiştir.

Anahtar Kelimeler
İmmobilize metal iyon afinite kromatografi, Albumin, Proteomik, Poli(glisidil metakrilat).

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INTRODUCTION

Serum proteins are important components for clinical therapeutical and diagnostical applications [1]. Albumin is the most abundant protein in blood plasma and makes up 57–71% of the total protein content in human plasma [2]. High abundance proteins tend to hide those of lower abundance which may prove to be informative disease markers [3,4]. The depletion of abundant serum proteins provides very important information in the early period of diseases [5]. Albumin is commonly isolated from human plasma by Cohn's method which is not highly spesific and can yield partially denaturated proteins. This method is the oldest method of industrial fractionation of proteins [6,7].

Recently, immobilized metal ion affinity chromatography (IMAC) is an alternative technique to Cohn's fractionation. IMAC has been used as a widespread separation technique that facilitates the isolation and purification of proteins [8-10]. IMAC is based on the formation of coordinate bonds between immobilized metal ions and the side chains of amino acids on the target protein surface [11]. This technique is especially convenient for the separation of biomolecules such as proteins and peptides owing to ligand stability, high protein binding capacity, mild elution conditions, simple regeneration and low costs [12-15]. The retention of protein on IMAC supports is affected by a diverse range of variables such as the surronding chemical environment, the character of the chelating group and metal ions specificity [16-18].

Bovine serum albumin (BSA) is generally used due to its low-cost and easy availability, binding properties and structural homology with human serum albumin [19,20]. In this study, iminodiacetic acid was proposed as a metal chelating ligand for use in the IMAC of BSA. Poly(glycidyl methacrylate) [p(GMA)] monosize microspheres were obtained by dispersion polymerization of GMA. Then Cu(II) ions were chelated on the p(GMA)-IDA microspheres which were used for BSA adsorption from aqueous solutions. The monosize microspheres were characterized by FTIR, SEM, elemental analysis. The adsorption conditions (i.e., pH, protein concentration, ionic strength, time, temperature) were investigated.

MATERIALS AND METHOD

Materials

BSA (molecular weight 66 kDa) was purchased from Sigma (Munich, Germany). Iminodiacetic acid (IDA) was purchased from Sigma (St Louis, USA). Glycidyl methacrylate (GMA) was obtained from Fluka (Buchs, Switzerland). Azobisisobutyronitrile (AIBN) was obtained from Fluka (Switzerland). Poly(vinyl pyrolidone) (PVP K-30, Mw: 40 000) was obtained from Aldrich Chemical (USA).

All other chemicals used were reagent grade from Merck A.G. (Darmstadt, Germany) unless otherwise noted. All water used in the adsorption experiments was purified using a Barnstead (Dubuque, IA, USA) 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 monosize p(GMA) microspheres

Monosize p(GMA) microspheres were prepared as described elsewhere [21]. The dispersion polymerization was performed in a sealed polymerization reactor equipped with temperature control system. The initiator (AIBN, 250 mg) was dissolved into the monomer phase which was composed of 40 mL GMA. The resulting medium was sonicated for 5 min at 200 W within an ultrasonic water bath (Bransonic 2200, England) for the complete dissolution of AIBN in the polymerization medium. Poly(vinyl pyrrolidone) (4.0 g) was dissolved in a mixture of ethanol (100 mL) and water (100 mL) placed in a polymerization reactor. The reactor content was stirred at 500 rpm during the monomer addition and the heating was started. Then the reactor was purged with bubbling nitrogen for 20 min. The polymerization was carried out in a shaking water bath at 65°C for 4 h. The reactor content was cooled down to room temperature after end of the polymerization and centrifuged at 5000 rpm for 10 min for the removal of dispersion medium. p(GMA) microspheres were redispersed within 10 mL of ethanol and centrifuged again under similar conditions. The microspheres were washed three times with ethanol. Finally, p(GMA)

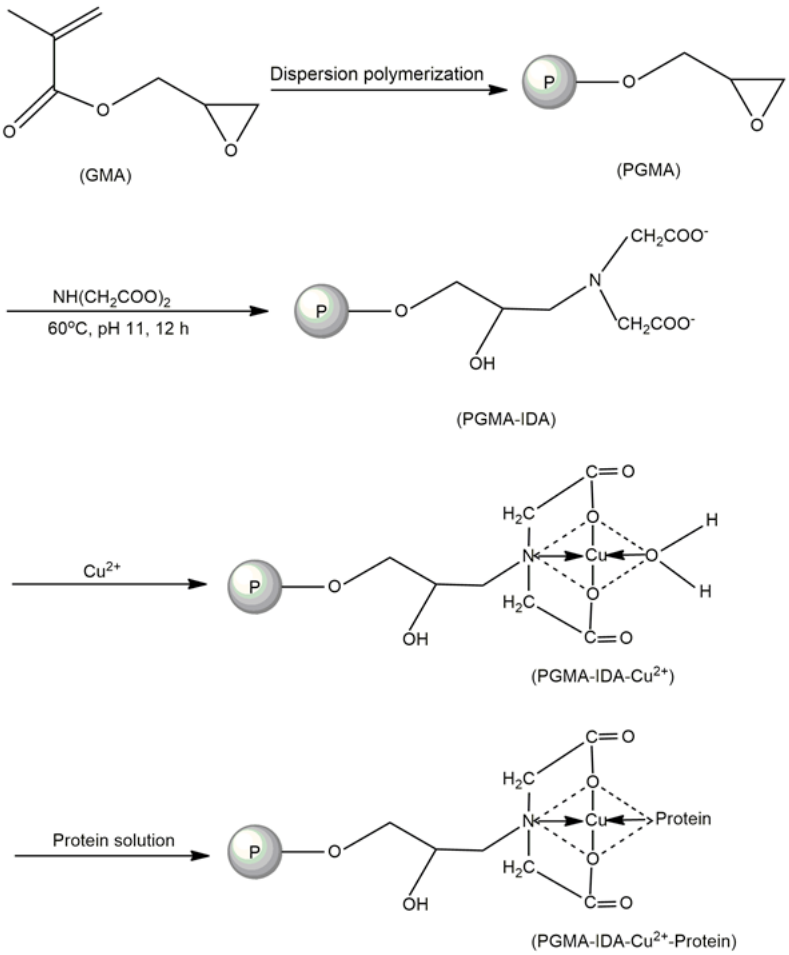

Figure 1. Preparation of p(GMA) metal-chelated microspheres.

microspheres were redispersed within 10 mL of water and stored at room temperature.

Immobilization of IDA to p(GMA) microspheres

In order to immobilize IDA onto p(GMA) microspheres, p(GMA) microspheres were treated with the reaction mixture (50 mL of 0.8 g IDA + 2.0 M Na_2CO_3, pH 11) at 70°C for 12 h in a heating mantle under mild stirring. After the end of the reaction, the microspheres were washed with 5% acetic acid and deionized water until the rinse water was neutral. Then, the blocking of unreacted epoxy groups with 2.0 M ethylene diamine at pH 10 for 16 h.

To remove non-specifically bound IDA molecules, the microspheres were first washed with deionized water and were sonicated with methanol for 2 h in an ultrasonic bath and finally washed with deionized water again. IDA immobilized p(GMA) microspheres were stored at 4°C with 0.02% sodium azide until use.

Chelation of Cu(II) Ions.

The IDA immobilized p(GMA) microspheres (1.0 g) were treated with aqueous solution containing 30 ppm Cu(II) ions (50 mL, pH 4.1) . The microsphere suspensions were stirred for 24 h at room temperature. The concentration of chelated Cu(II) ions in the supernatants can be determined by atomic absorption spectrophotometer (AAS; Analyst 800, Perkin Elmer, USA). As mentioned before, the experimental procedure is depicted in Figure 1. Monosize p(GMA)-IDA-Cu(II) microspheres were stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

BSA adsorption on monosize p(GMA) microspheres

Adsorption of BSA onto the monosize p(GMA)-IDA-Cu(II) microspheres was performed at 25°C for 2 h in a batch system. The effects of pH, BSA concentration, ionic strength, time and temperature on adsorption were explored. The BSA concentration was measured at 280 nm using UV/Vis spectrophotometer (Model 1601, Shimadzu, Tokyo, Japan). The amount of adsorbed BSA onto the monosize p(GMA)-IDA-Cu(II) microspheres was measured by using the initial and final concentrations of protein. BSA desorption from the microspheres was carried out by using 1.0 M NaCl solution. All measurements were carried out in triplicate and the average values are reported.

Characterization of Monosize Microspheres

The amount of attached IDA was calculated from the stoichiometry ratio of nitrogen using an elemental analysis instrument (Leco, CHNS-932, U.S.A.). To identify the functional groups of IDA attachment on p(GMA) microspheres, Fourier transform infrared (FTIR) measurements were performed on a Shimadzu FTIR 8000 series spectrometer in normal transmission mode using a KBr detector over the range of 400–4000 cm^{-1}. The surface morphology and particle size of the microspheres were investigated using scanning electron microscopy (JEOL JEM 1200 EX, Jeol, Tokyo, Japan). The perchloric acid titration method was used for determination of the amount of epoxy group in the p(GMA) microspheres. Therefore the monosize p(GMA) microspheres were dispersed in tetraethylammonium bromide (0.1 M) in acetic acid solution and titrated with perchloric acid solution (0.1 M) until the color of crystal violet indicator changed form violet to blue-green.

BSA adsorption-desorption studies

Adsorption of BSA onto the p(GMA)-IDA-Cu(II) microspheres was carried out in a batch system. The effects of pH, BSA concentration, ionic strength, time and temperature on adsorption were investigated. All BSA adsorption studies were performed for 120 min at 25°C. The amount of adsorbed BSA on the p(GMA)-IDA-Cu(II) microspheres was measured at 280 nm by UV/vis spectrophotometer. BSA desorption from the microspheres was performed with 1.0 M NaCl. Every measurements were carried out in triplicate and the average values are reported.

RESULTS AND DISCUSSION

Characterization studies

The surface morphology and particle size of the p(GMA) microspheres were analyzed by SEM. It can be seen that the p(GMA) microspheres are monodispersed microparticles with a diameter of 1.6 μm (Figure 2). Polydispersity index (PDI) value of poly(GMA) microspheres was calculated to be around 1.006. FTIR spectrums were undertaken to determine the structure of the poly(GMA) and the IDA-attached poly(GMA) microspheres (Figure 3). The FTIR spectra of IDA-attached poly(GMA) microspheres showed characteristic peaks that appear at 2950 cm^{-1} (CH_3 stretching vibration), 2132 cm^{-1} (C-N stretching vibration), and 1731 cm^{-1} (carbonyl stretching vibration). The N-H peak that appears at 3626 cm^{-1} is associated with the IDA. These data confirmed that the monosize microspheres were modified with functional groups IDA.

Metal-chelating ligand IDA is covalently attached on monosize p(GMA) microspheres via the reaction between the epoxide groups of the GMA and the primer amine groups of the IDA. The amount IDA surface density was found 264.29 μmol/g polymer using nitrogen stoichiometry. The content of epoxy groups on the surface of the monosize p(GMA) microspheres was determined according to the pyridine-HCl titration with 0.1 M NaOH (6.0 mmol/g polymer). The amount of chelated Cu(II) ions was 225.30 μmol/g as determined by AAS.

Effect of pH on adsorption capacity

The amount of adsorbed BSA onto the p(GMA)-IDA-Cu(II) microspheres as a function of pH is presented in Figure 4. For this aim, acetate and phosphate buffers were used for pH 4.0–5.0 and 6.0–8.0 respectively. The maximum value for the adsorption capacity was obtained at pH 5.0. The amount of adsorbed BSA decreased significantly at pH values less and greater than 5.0. The results may depend on the ionization state of BSA and repulsive electrostatic forces between adsorbed

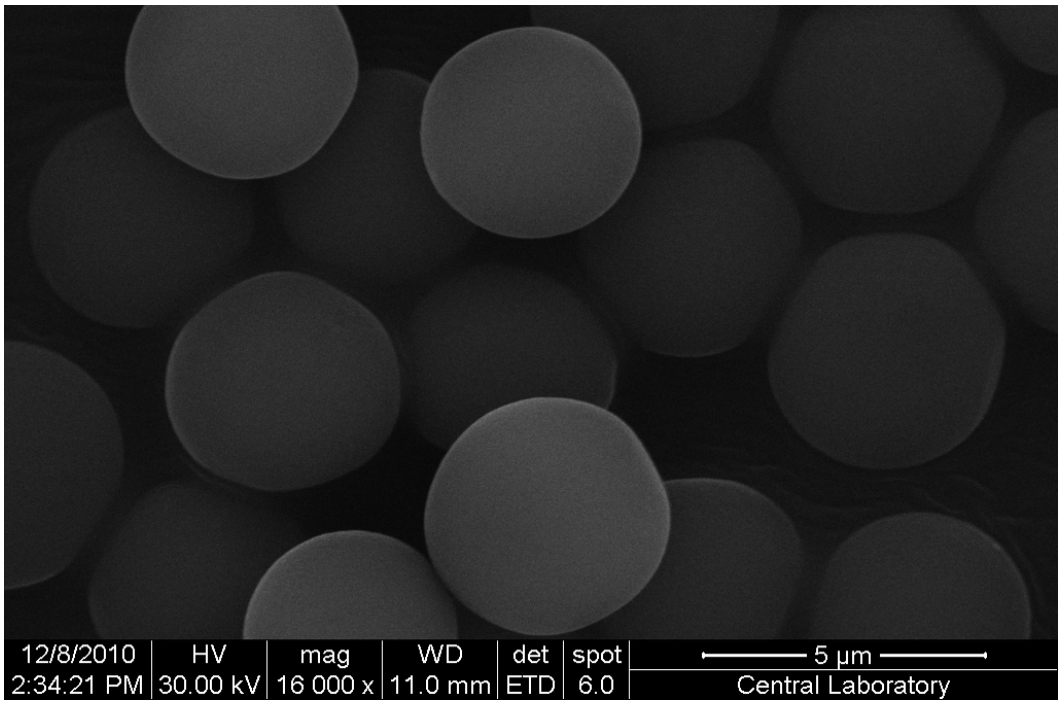

Figure 2. SEM image of monosize p(GMA) microspheres.

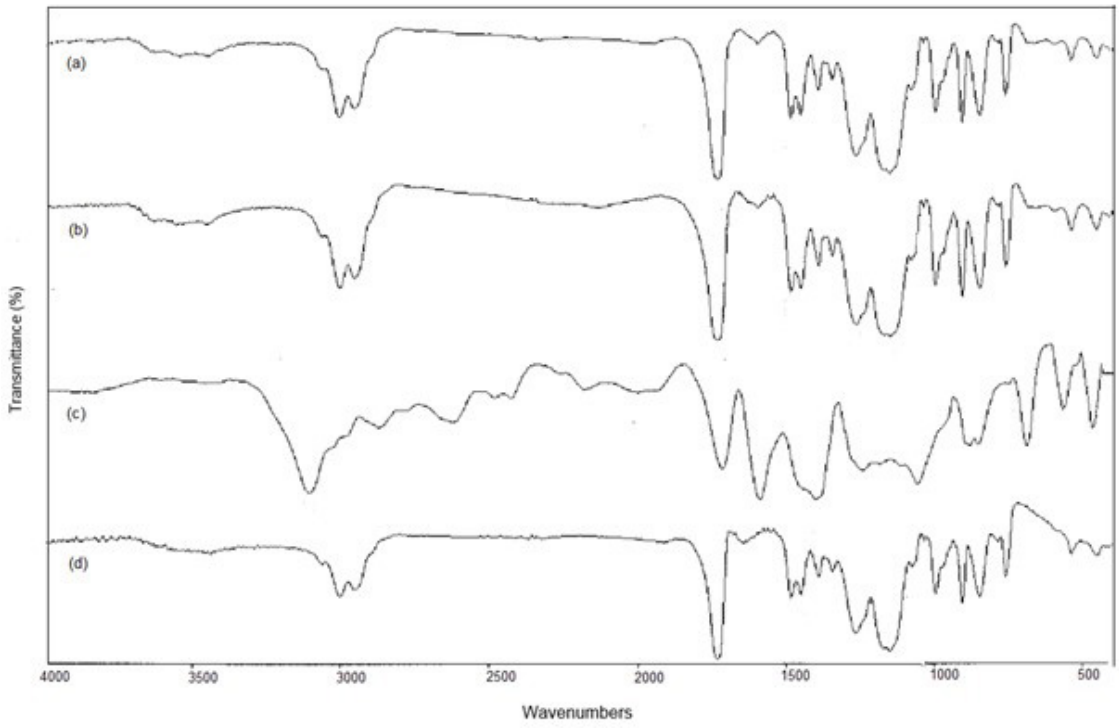

Figure 3. FTIR spectra of p(GMA) (a), p(GMA)-IDA-Cu(II) (b), IDA (c), p(GMA)-IDA (d) microspheres.

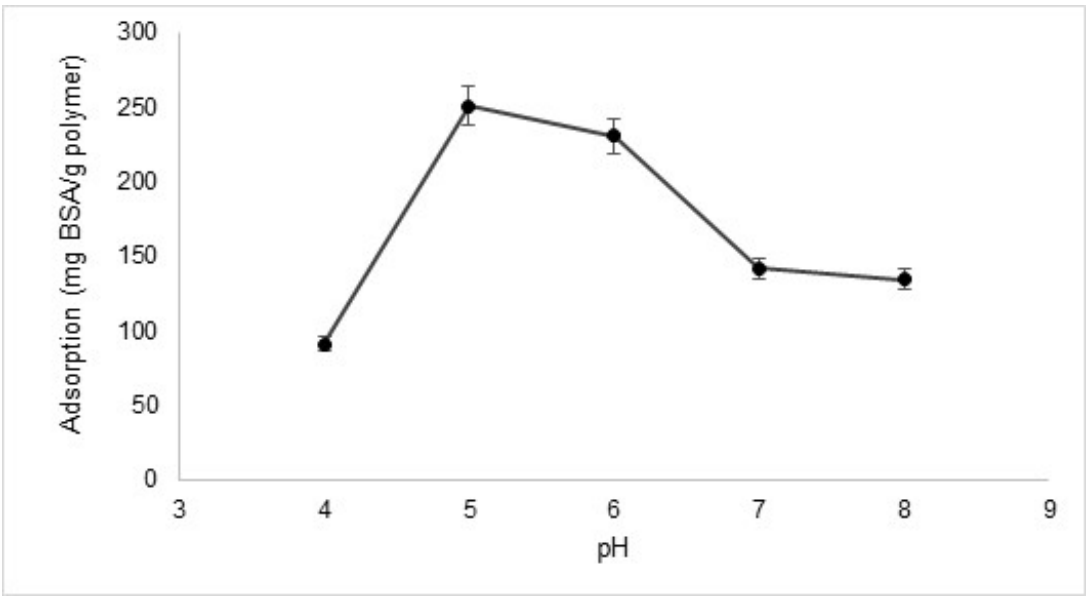

Figure 4. Effect of pH on BSA adsorption (BSA concentration: 2.0 mg/mL; T: 25°C).

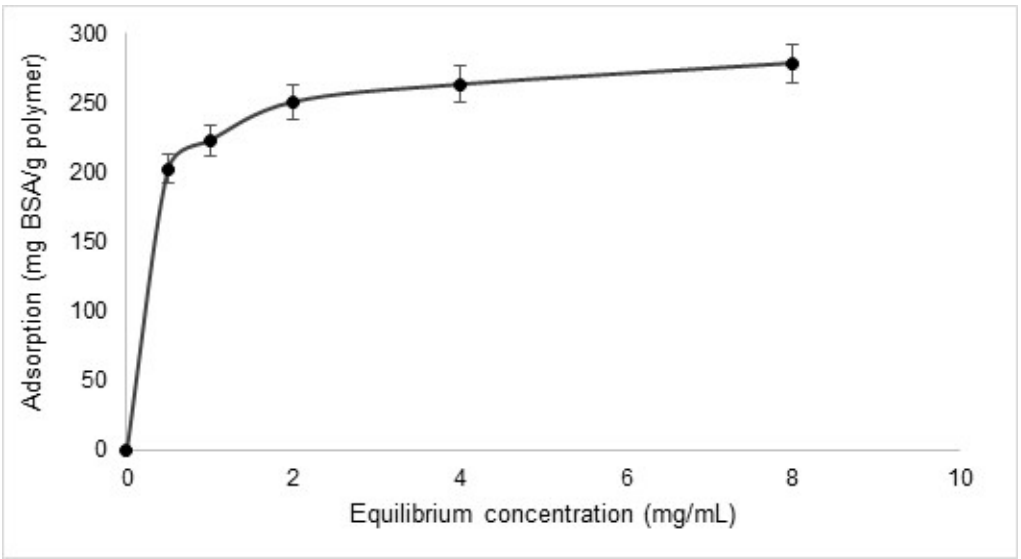

Figure 5. Effect of equilibrium BSA concentration (pH: 5.0; T: 25°C).

BSA molecules and the chelated Cu(II) ions on p(GMA)-IDA microspheres. The optimal pH value was accepted as pH 5.0 which was kept constant for further studies.

Effect of time on adsorption capacity with changing protein concentration

Figure 5 indicates that the effects of time on amount of BSA adsorbed on the monosize p(GMA)-IDA-Cu(II) microspheres by changing BSA concentration. As seen in the figure, very high adsorption rates are observed at the beginning of adsorption and then, the rate of adsorption decreases within 120 min with the increase of time, plateau values are gradually reached which shows saturation of the active binding sites on the p(GMA)-IDA-Cu(II) microspheres.

Effect of equilibrium BSA concentration

Figure 6 shows that the initial concentrations of BSA in the aqueous phase were changed between 0.5-8.0 mg/mL. The equilibrium adsorption of BSA increased with increase in the amount of BSA in buffer solution. Maximum adsorption capacity

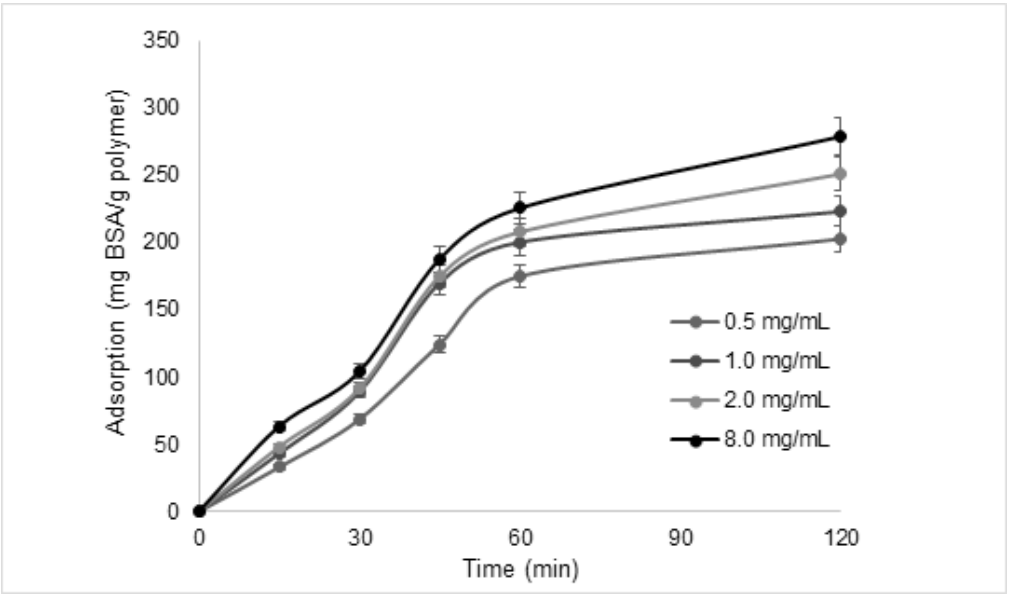

Figure 6. Effect of BSA concentration on BSA adsorption (pH: 5.0; T: 25°C).

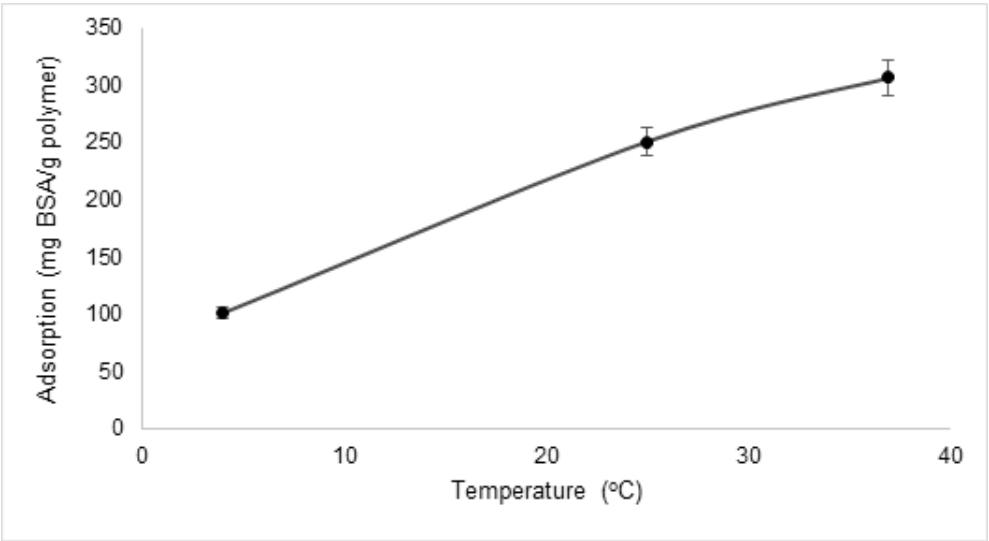

Figure 7. Effect of temperature on BSA adsorption (BSA concentration: 2.0 mg/mL; pH: 5.0).

of the p(GMA)-IDA-Cu(II) microspheres was found 278.6 mg/g polymer for 8 mg/mL of BSA at pH 5.0. The increase of protein adsorption might be due to the high affinity between BSA and Cu(II)-chelated monosize p(GMA) microspheres.

Effect of temperature and ionic strength on adsorption capacity

The effect of temperature on BSA loading capacity of p(GMA)-IDA-Cu(II) microspheres is presented in the range of 4°C–37°C in Figure 7. Adsorption of BSA on the p(GMA)-IDA-Cu(II) microspheres was crucially increased with increasing temperature in order to the hydrophobic interaction plays an essential role in the adsorption.

The effect of ionic strength on BSA adsorption is shown in Figure 8 The adsorption capacity decreased with increasing NaCl concentration. The adsorption of BSA was decreased by about 65% while the NaCl concentration increased from 0 to 0.1 M. The increase in the ionic strength causes the decrease in adsorption capacity of BSA onto the p(GMA)-IDA-Cu(II) surface.

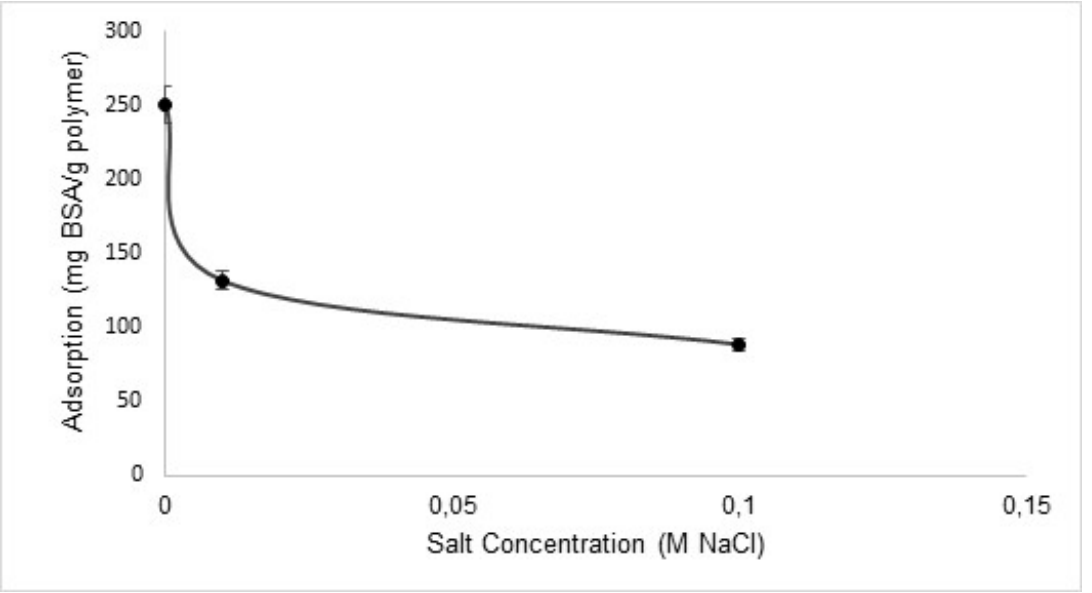

Figure 8. Effect of NaCl concentration on BSA adsorption. (BSA concentration: 2.0 mg/mL; pH: 5.0; T: 25°C).

This situation can be attributed to the repulsive electrostatic interactions between the p(GMA)-IDA-Cu(II) microspheres and BSA molecules.

CONCLUSION

IMAC is one of the most successful and efficient technique for the separation and enrichment of biomolecules [22]. Using this technique, removal of BSA in a rapid single-step is carried out by our metal-chelated microspheres. Compared to other affinity separation technologies IMAC possesses significant advantages such as, ligand stability, high protein loading, gentle elution conditions, simple regeneration and inexpensive [23]. In this study, microspheres were used as metal-chelated affinity support and iminodiacetic acid was used as the metal coordinating agent. Cu(II) ions were chelated via IDA groups on monosize p(GMA) microspheres for affinity binding of BSA. The maximum BSA adsorption capacity by using metal chelated microspheres was found to be 278.6 mg/g polymer. The metal-chelated microspheres can be offered the promising approach for current proteomic studies and drug-target screens.

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