Comparison of Metal Chelate Affinity Sorption of BSA onto Dye/Zn(II)-Derived Poly(ethylene glycol dimethacrylate–hydroxyethyl methacrylate) Microbeads

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ABSTRACT: Poly(ethylene glycol dimethacrylate–hydroxyethyl methacrylate) [poly(EGDMA–HEMA)] microbeads in the size range of 150–200 μm were produced by a modified suspension copolymerization of EGDMA and HEMA. The dyes (Congo red, Cibacron blue F3GA, and alkali blue 6B) were covalently immobilized; then, Zn(II) ions were incorporated within the microbeads by chelation with the dye molecules. The maximum amounts of dye loadings were 14.5 μmol/g, 16.5 μmol/g, and 23.7 μmol/g for Congo red, Cibacron blue F3GA, and alkali blue 6B, respectively. Different amounts of Zn(II) ions (2.9–53.8 mg/g polymer) were incorporated on the microbeads by changing the initial concentration of Zn(II) ions and the pH of the medium. Bovine serum albumin (BSA) adsorption onto dye/Zn(II)-derived microbeads containing Congo red, Cibacron blue F3GA, and alkali blue 6B was investigated. The maximum BSA adsorptions onto the dye/Zn(II)-derived microbeads from aqueous solutions containing different amounts of BSA were 159 mg/g, 122 mg/g, and 93 mg/g for the Congo red, Cibacron blue F3GA, and alkali blue 6B dyes, respectively. The maximum BSA adsorptions were observed at pH 6.0 in all cases. Desorption of BSA molecules was achieved by using 0.025*M* EDTA (pH 4.9). High desorption ratios (more than 93% of the adsorbed BSA) were observed in all cases. It was possible to reuse these novel metal chelate sorbents without significant losses in their adsorption capacities. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **65:** 2085–2093, 1997

Key words: metal chelate adsorption, affinity microbeads, albumin adsorption, poly(EGDMA-HEMA)

INTRODUCTION

The chromatographic separation of proteins is important not only for analysis but also for separating them in large-scale industries, such as the food and drug industries. Several chromatographic methods for separating proteins have been developed. Among these methods, the metal chelate affinity technique has, in the past decade, found wide acceptance and application in the recovery of proteins mainly owing to their high selectivity. Metal chelate affinity chromatography introduces an entirely new basis for separating proteins given their affinity for transition metal ions. Many transition metal ions (e.g., zinc and copper) can coordinate with amino acids such as histidine, cysteine, and tryptophan via electron donor groups on the amino acid side chains.[1–4]

The interaction of metal chelates with proteins containing surface-exposed amino acids such as histidine, cysteine, and tryptophan was first de-

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scribed by Porath et al.[5] Subsequent studies have shown the wide applicability of the technique and consistency of the methodology. The plasma proteins α_2-macroglobulin and α_1-proteinase inhibitor, for example, have been purified to homogeneity on zinc chelate columns.[6,7] Metal chelate affinity chromatography has been used to provide immunologically and physicochemically pure α_2-HS glycoprotein from plasma.[8,9] Plasminogen activators from both normal tissue (human uterus) and human melanoma cells have been isolated by metal chelate affinity chromatography,[10,11] as have nucleoside diphosphatase,[12] human lactoferrin,[13] lectin,[14] interferon,[15] and carboxy-peptidase B.[16]

In our recent studies, we developed a series of swellable polymeric microbeads containing different chelating dyes and metal ions and evaluated them as affinity sorbents for heavy metal removal and protein separation.[17–21] The purpose of the present study was to further extend our earlier attempts to use metal affinity chromatography for adsorption of bovine serum albumin (BSA). We used three reactive dye ligands (Congo red, Cibacron blue F3GA, and alkali blue 6B) and Zn(II) ions (in chelate form). We selected BSA as a model protein. In this article we present BSA adsorption/desorption properties of these dye/Zn(II)-derived microbeads.

EXPERIMENTAL

Production of Dye-Derived Microbeads

Poly(EGDMA–HEMA) microbeads were selected as the base material for the synthesis of a chromatographic affinity matrix and produced by a modified suspension polymerization of the respective monomers [i.e., ethylene glycol dimethacrylate (EGDMA, Rohm, Germany) and 2-hydroxyethyl methacrylate (HEMA, Sigma, USA)] in an aqueous media.[17–19] Benzoyl peroxide (BPO) and poly(vinyl alcohol) [PVAL (MW: 100.000, 98% hydrolyzed, Aldrich Chemical Co., USA)] were used as the initiator and the stabilizer, respectively. Toluene (Merck, Germany) was selected as the diluent and used as received. The dispersion medium was distilled water. To produce polymeric microbeads with about a 150–200-μm diameter and with a narrow size distribution, the amounts of EGDMA, HEMA, toluene, water, BPO, and PVAL used were 8 ml, 4 ml, 12 ml, 50 ml, 0.06 g, and 0.2 g, respectively. Polymerizations were carried out at an agitation rate of 600 rpm at 65°C for 4 h and at 90°C for 2 h. After cooling, the polymeric microbeads were separated from the polymerization medium by filtration, and the residuals (e.g., unconverted monomer and toluene) were removed by a cleaning procedure given in detail elsewhere.[22]

The dyes (Congo red, Cibacron blue F3GA, and alkali blue 6B) were purchased from BDH (UK). Three grams of poly(EGDMA–HEMA) microbeads were magnetically stirred (at 400 rpm) in a sealed reactor at a constant temperature of 80°C for 4 h with 100 ml of the dye aqueous solution containing 4.0 g NaOH. To change the immobilized dye concentration on the microbeads, the initial concentration of the dye was varied between 0.1 and 4.0 mg/ml. After derivation, the dye-derived microbeads were filtered and washed with distilled water and methanol several times until all the physically attached dye molecules were removed. The dyed microbeads were stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

The amounts of immobilized dye on the microbeads were determined by using an elemental analysis instrument (Leco, CHNS-932, USA). The amount of dye on the microbeads was obtained from these data by considering the nitrogen and sulfur stoichiometries.

The leakage of the dye from the dye-derived microbeads was investigated within the media containing NaCl at ionic strength 0.01 and at the selected pH in the range of 4.0–8.0. Note that these media were also used in the BSA adsorption experiments described in the Results and Discussion section. Dye leakage was also determined in the medium containing 0.025*M* EDTA at pH of 8.0, which was the medium used in the BSA desorption experiments. The medium with the dye-derived microbeads was stirred for 24 h at room temperature. Then the polymeric microbeads were separated from the medium, and the dye concentrations were measured in the liquid phase by spectrophotometry at 497 nm for Congo red, 599 nm for alkali blue 6B, and 630 nm for Cibacron blue F3GA.

Zn(II) Incorporation

Chelates of dye-derived microbeads with Zn(II) ions were prepared as follows: One hundred milligrams of dye-derived (Congo red, Cibacron blue F3GA, and alkali blue 6B) microbeads were mixed with aqueous solutions containing 1–400 ppm

Zn(II) ions at room temperature. The pH values of the medium were changed over a range of 3.0–7.5 (adjusted with universal buffer solution). A 1000-ppm atomic absorption standard solution (containing 10% concentrated HNO_3) was used as the source of Zn(II) ions. The flasks were agitated magnetically at 600 rpm for 1 h (sufficient to attain equilibrium). The concentration of the Zn(II) ions was determined with an atomic absorption spectrophotometer (GBC 932 AA, Australia).[17–20]

Zn(II) leakage from the dye/Zn(II)-derived microbeads was investigated with media containing NaCl (ionic strength 0.01) and having pH values in the range of 4.0–8.0. The microbead suspensions were stirred 24 h at room temperature. The Zn(II) ion concentration was then determined in the supernatants using an atomic absorption spectrophotometer.

BSA Adsorption/Desorption

Bovine serum albumin (BSA, lyophilized, Fraction V, Sigma, USA) was selected as a model protein. The BSA adsorption on the plain, the dye-attached, and the dye/Zn(II)-derived poly(EGDMA–HEMA) microbeads was studied in the media with respect to batch at different pH values. The pH of the adsorption medium was changed between 4.0 and 8.0 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, and 0.1*M* NH_4OH–NH_4Cl for pH 8.0). The ionic strength of the adsorption media was 0.01 (adjusted by using NaCl). The BSA initial concentration was varied between 0.5–7.0 mg/ml. In a typical adsorption experiment, BSA was dissolved in 25 ml of buffer solution containing NaCl, and 200 mg of microbeads were added. The adsorption experiments were conducted for 2 h at 25°C and at a stirring rate of 100 rpm. At the end of this equilibrium period, the microbeads were separated from the solution by centrifugation. The albumin adsorption capacity was determined by measuring the initial and final concentrations of BSA within the adsorption medium spectrophotometrically at 280 nm.[23]

The BSA desorption experiments were performed in a buffer solution containing 0.025*M* EDTA at pH 4.9. The BSA-adsorbed microbeads were placed in the desorption medium and stirred for 1 h at 25°C at a stirring rate of 100 rpm. The final BSA concentration within the desorption medium was determined by spectrophotometry. In the case of the Zn(II)-incorporated sorbents, desorption of Zn(II) ions was also measured in the desorption media by means of an atomic absorption spectrophotometer. The desorption ratio was calculated from the amount of BSA adsorbed on the microbeads and the amount of BSA desorbed.

To ensure the reusability of the dye/Zn(II)-derived poly(EGDMA–HEMA) microbeads, the BSA adsorption-desorption cycle was repeated three times by using the same polymeric microbeads. After each experiment, the Zn(II) was stripped with 0.025*M* EDTA at pH 4.9, and the Zn(II) loading operation was repeated.

RESULTS AND DISCUSSION

Characteristics of Poly(EGDMA–HEMA) Microbeads

Details of the production and characterization of poly(EGDMA–HEMA) microbeads were given in our previous articles.[17–21] The microbeads produced by the recipe under the conditions given in the Experimental section are highly swellable (due to HEMA) in aqueous media (the swelling ratio is 55%), and are in the size range of 150–200 μm (i.e., swollen size). They are hard and very strong because of the highly crosslinked structure (due to EGDMA), and therefore they are suitable for column applications in chromatographic separations. A representative optical photograph of poly(EGDMA–HEMA) microbeads is presented in Figure 1.

In the present study, we attempted to prepare specific affinity sorbents containing three different dyes (Congo red, Cibacron blue F3GA, and alkali blue 6B) and Zn(II) ions (in chelate form) for metal chelate affinity adsorption of BSA. Chemical structures of these reactive dyes are given in Figure 2. As can be seen, their chemical structures are quite different from each other and contain several active points (amino, azo, sulfonyl, and triazine groups, as indicated in Fig. 2) for chelation with Zn(II) ions.

The dye molecules were covalently incorporated in the poly(EGDMA–HEMA) microbeads. Cibacron blue F3GA is a widely used dye ligand (even in the commercial sorbents). It is accepted that ether linkages are formed between the reactive triazine ring of the dye and the hydroxyl groups of the sorbent (like HEMA groups in our case).[24] In the case of Congo red and alkali blue 6B, covalent bonds are formed as a result of the condensation reactions between the aromatic

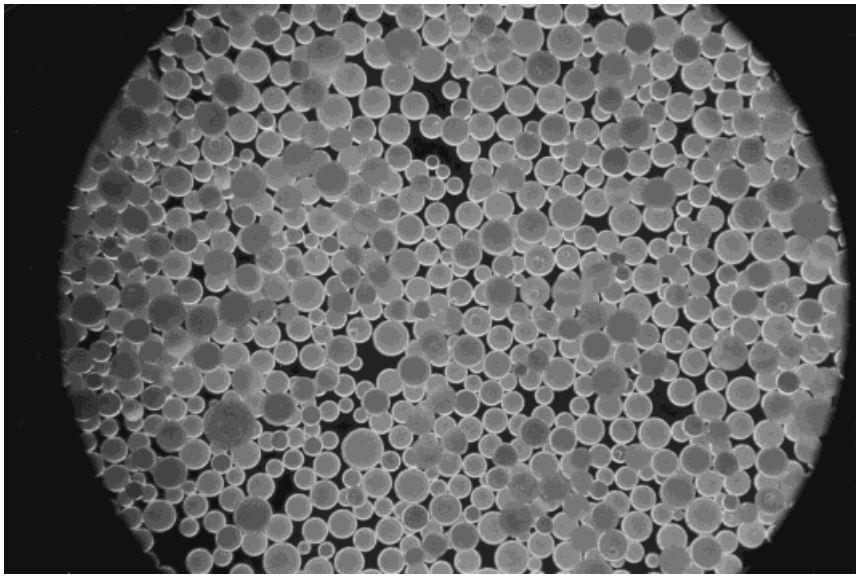

Figure 1 Optical photograph of poly(EGDMA–HEMA) microbeads.

amine groups of the dyes and the hydroxyl groups of the HEMA, as mentioned in our related articles.[17–21] Note that the dye-derived microbeads were extensively washed as described in the Experimental section to prevent leakage from any of the dye-derived microbeads and in any media used in the adsorption-desorption steps. The visual observations (the color of the microbeads), elemental analysis, and FTIR spectra, which were already reported in our previous articles, ensured incorporation of dye molecules.[17–21]

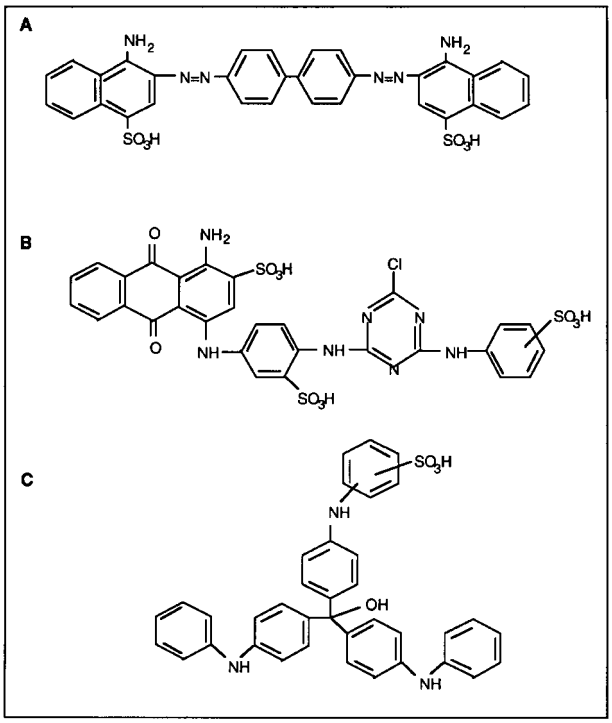

Figure 2 Structure of (a) Congo red, (b) Cibacron blue F3GA, and (c) alkali blue 6B.

Zn(II) Incorporation

Influence of Dye Loading on Zn(II) Adsorption

The initial concentration of each dye in the dye-derived medium was changed in the range of 0.1–4.0 mg/ml, and the extent of dye loading (i.e., the amount of immobilized dye per gram of the microbeads) was determined by elemental analysis. Figure 3 shows the influence of dye concentra-

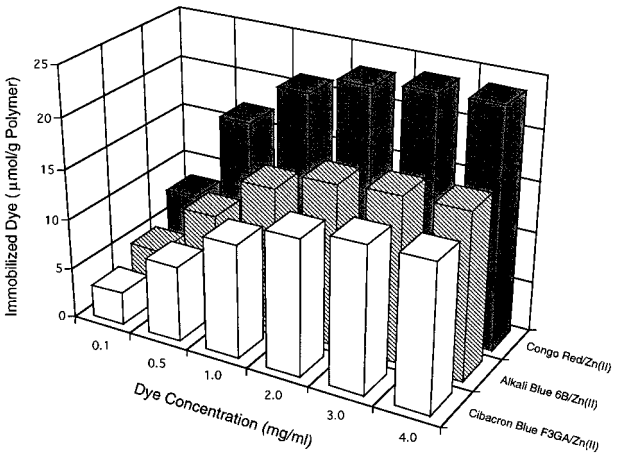

Figure 3 Influence of dye concentration on dye incorporation.

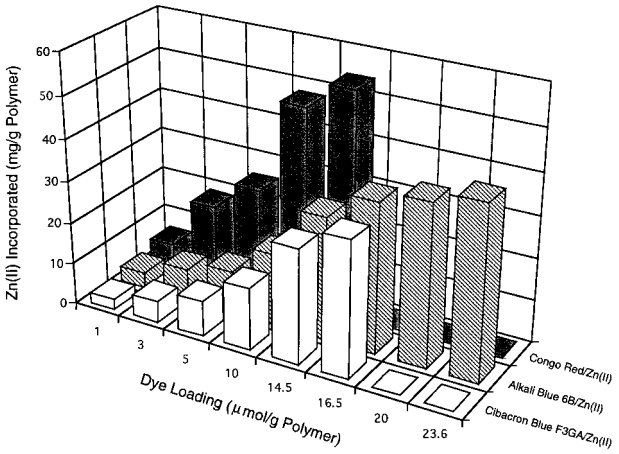

Figure 4 Influence of dye loadings on Zn(II) incorporation [Zn(II) initial concentration: 400 ppm; pH: 6.5; temperature: 20°C].

tion on dye loading. Dye loading increased with the initial concentration of the dye and then reached a plateau around a 2-mg/ml dye concentration. Maximum dye loadings were 14.5, 16.5, and 23.6 μmol dye/g polymer for Congo red, Cibacron blue F3GA, and alkali blue 6B, respectively. The order of dye loading on and in the microbeads was as follows: alkali blue 6B > Cibacron blue F3GA > Congo red.

Figure 4 shows the influence of dye loading on Zn(II) incorporation. When the number of dye molecules on the microbeads increased (higher dye loadings), the amount of Zn(II) incorporation on the dye-derived microbeads also increased. The maximum amount of Zn(II) incorporation on the dye-derived microbeads for Congo red, alkali blue 6B, and Cibacron blue F3GA was 53.8, 41.4, and 32.4 mg Zn(II)/g polymer, respectively. The order of Zn(II) incorporation on the microbeads was as follows: Congo red > alkali blue 6B > Cibacron blue F3GA. Most probably, Congo red molecules on the derived microbeads were in a more favorable form for chelation with Zn(II) ions than alkali blue 6B and Cibacron blue F3GA molecules.

It is agreed that the ligand molecules should be immobilized on the carrier matrices in such a way that their geometrical structures in the immobilized form will be available to the molecules with which they will interact.[25] Most probably, Congo red molecules were immobilized on the microbeads in a more favorable form than those of the other dyes, which resulted in higher Zn(II) incorporation.

Influence of Zn(II) Initial Concentration on Zn(II) Incorporation

Figure 5 shows the influence of Zn(II) ion concentration on the amount of Zn(II) ions incorporated on the dye-derived microbeads. The amount of Zn(II) ions chelated per unit mass of the polymer (i.e., adsorption capacity) increased first with the initial concentration of Zn(II) ions and then reached a plateau value representing saturation of the active points available for specific Zn(II) ions. These plateau values were around 300 ppm for all dyes. The maximum Zn(II) adsorption values were 53.8, 41.4, and 32.4 mg Zn(II)/g polymer for Congo red, Cibacron blue F3GA, and alkali blue 6B, respectively.

Note that the nonspecific adsorption of Zn(II) ions onto the plain poly(EGDMA–HEMA) microbeads was relatively low [about 0.4 mg Zn(II)/g polymer]. No ion exchange takes place nor is there any evidence of chelate forming groups on the plain poly(EGDMA–HEMA) microbeads. This adsorption may be due to diffusion of Zn(II) ions into the swollen matrix of the microbeads.

Influence of Medium pH on Zn(II) Incorporation

It is well known that pH influences metal ion adsorption on both nonspecific and specific sorbents. In the absence of chelating agents, the hydrolysis and precipitation of the metal ions are affected by the concentration and form of the soluble metal species. The solubility of metal ions is governed by hydroxide or carbonate concentration.[26] Hydrolysis of Zn(II) ions becomes significant at a pH of approximately 7.5. Therefore, in our study, in or-

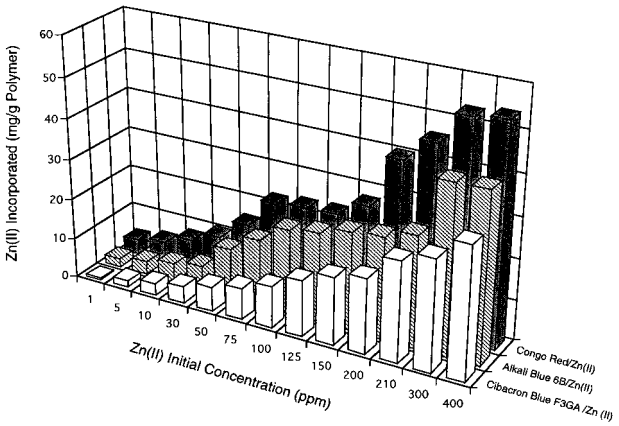

Figure 5 Influence of Zn(II) ions concentration on Zn(II) incorporation (dye loading: 14.5 μmol Congo red/g; 16.5 μmol Cibacron blue F3GA/g; 23.6 μmol alkali blue 6B/g; medium pH: 6.5; temperature: 20°C).

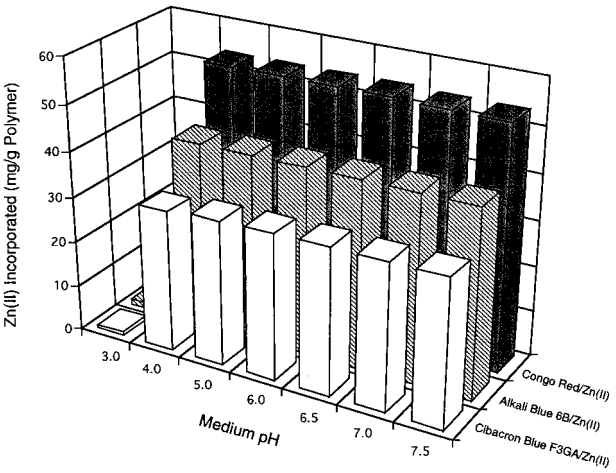

Figure 6 Influence of medium pH on Zn(II) adsorption (dye loadings: 14.5 μmol/g for Congo red; 16.5 μmol/g for Cibacron blue F3GA; 23.6 μmol/g alkali blue 6B; Zn(II) initial concentration: 400 ppm; temperature: 20°C).

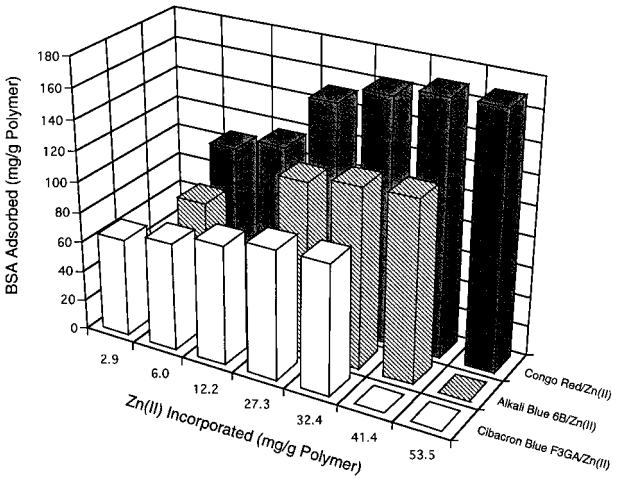

Figure 7 Influence of Zn(II) loading on BSA adsorption [dye loading: 14.5 μmol/g; BSA initial concentration: 5.0 mg/ml; pH: 6.0; ionic strength: 0.01 (adjusted with NaCl); and temperature: 20°C].

der to establish the effect of pH on the incorporation of Zn(II) ions onto the dye-derived microbeads, we repeated the batch equilibrium adsorption studies at different pH values in the range of 3.0–7.5. Figure 6 shows the influence of pH on Zn(II) incorporation on the dye-derived microbeads. It can clearly be seen that there was almost no interaction between the immobilized dye molecules and Zn(II) ions at pH 3.0, whereas incorporations are observable at pH 4.0 and above. Most probably, the functional groups on the immobilized dye molecules ionize and interact with Zn(II) ions above pH 4.0.

BSA Adsorption

In the second part of the study, the amounts of incorporated Zn(II) ions, the influence of BSA initial concentration and the medium pH upon the BSA adsorption on the dye/Zn(II)-derived microbeads, and desorption of BSA and reusability were investigated and are separately discussed in the following subsections.

Influence of Zn(II) Loading on BSA Adsorption

BSA adsorption capacities of the dye/Zn(II)-derived poly(EGDMA–HEMA) microbeads containing different amounts of Zn(II) (between 2.9 and 53.8 mg Zn(II)/g polymer) were investigated at pH 6.0. The initial concentration of BSA in the incubation solution was 5.0 mg/ml. Figure 7 shows the influence of Zn(II) incorporation on BSA adsorption. When the amount of Zn(II) on the microbeads increased, the amount of BSA adsorbed first increased and then reached an almost constant value. This value may be the maximum amount of BSA that can be packed on the surface, owing to steric constraints.

Influence of BSA Initial Concentration on BSA Adsorption

Figure 8 shows the BSA-adsorption curves for the dye/Zn(II)-derived microbeads. In all cases, BSA

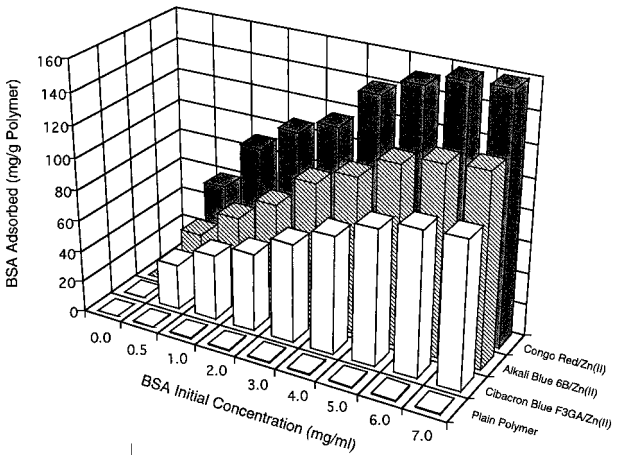

Figure 8 Influence of BSA initial concentration on BSA adsorption [dye loading: 14.5 μmol/g; Zn(II) loading: 27.3 mg/g; pH: 6.0; ionic strength: 0.01 (adjusted with NaCl); and temperature: 20°C].

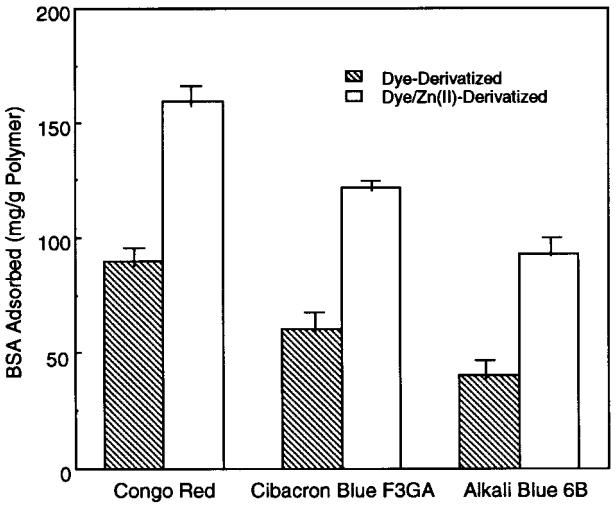

Figure 9 Comparison of BSA adsorption onto dye-attached and dye/Zn(II)-derived microbeads [dye loading: 14.5 μmol/g; Zn(II) loading: 27.3 mg/g; pH: 6.0; ionic strength: 0.01 (adjusted with NaCl); and temperature: 20°C].

adsorption first increases and then reaches a plateau (the first plateau may be observed around a 2-mg/ml initial BSA concentration), which may be considered a typical example of the coverage of the sorbent surface with a monolayer of BSA molecules. However, as can clearly be seen, especially in the curve of Congo red-derived microbeads, another step starts around a BSA initial concentration of 3 mg/ml. Adsorption increases again after this value but reaches the second plateau, which may be an indication of the formation of the second BSA layer on the microbeads. Once again, the maximum BSA adsorptions for Congo red, Cibacron blue F3GA, and alkali blue 6B were 159, 122, and 93 mg BSA/g polymer, respectively, which are comparatively in high comparison with the related literature data.[21,27–31] The order of BSA adsorption on the dye/Zn(II)-derived microbeads is as follows: Congo red/Zn(II) > Cibacron blue F3GA/Zn(II) > alkali blue 6B/Zn(II).

It should be mentioned that the nonspecific BSA adsorption [adsorption on the plain poly(EGDMA–HEMA) microbeads] was very low (0.051 mg BSA/g polymer).

To observe the influence of dye grafting conditions on BSA adsorption, poly(EGDMA–HEMA) microbeads exposed to dye-grafting conditions without dye were also evaluated for BSA adsorption. The BSA adsorption observed was in the same amount (0.051 mg BSA/g polymer) as that detected for plain microbeads. It can be said that the poly(EGDMA–HEMA) microbeads are impervious to the harsh alkaline conditions used for dye grafting. No carboxylic moieties are likely to be produced by hydrolysis of the ester linkage of HEMA and hence they do not contribute to BSA adsorption. Therefore, it can be said that BSA molecules were adsorbed via derived dye-Zn(II) groups.

The BSA adsorption experiments were also performed using the dye-derived microbeads. The comparative results are given in Figure 9. As seen here, Zn(II) incorporation significantly increased the BSA adsorption capacity between 76 and 133%, which implies that Zn(II) incorporation plays an important role for adsorption of BSA, in keeping with the objective of this study.

Influence of pH on BSA Adsorption

Figure 10 shows the influence of medium pH on BSA-adsorption onto the dye/Zn(II)-derived microbeads. In all the cases investigated, the maximum adsorption of BSA was observed at pH 6.0. Significantly lower BSA adsorption capacities were obtained with all microbeads at lower and higher pH values. It has been shown that proteins have no net charge at their isoelectric points, and therefore the maximum adsorption from aqueous solutions is usually observed at their isolectric point.[32] The isoelectric pH of BSA is 5.0. In our case, the maximum adsorption pH was not 5.0 but rather shifted to higher pH values than the isoelectric point of BSA. This may be because of preferential interaction between BSA and dye/

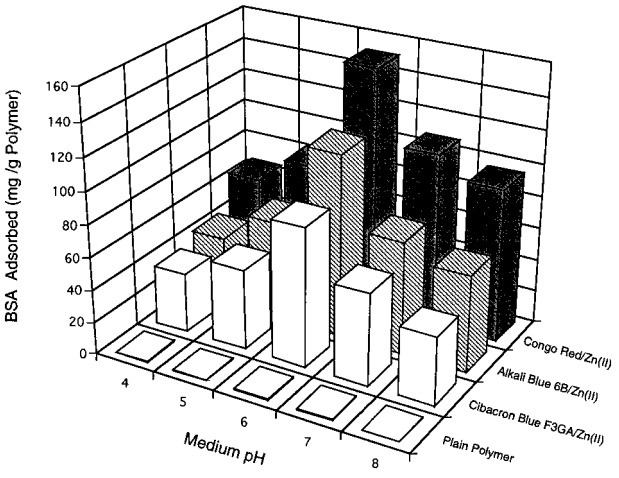

Figure 10 Effects of medium pH on BSA adsorption [dye loading: 14.5 μmol/g; Zn(II) loading: 27.3 mg/g; BSA initial concentration: 5.0 mg/ml; ionic strength: 0.01 (adjusted with NaCl); and temperature: 20°C].

Table I BSA Desorption from Dye/Zn(II)-Derived Microbeads

BSA Initial Concentration (mg/ml)	Desorption Ratio (%)		
	Congo Red–Zn(II)	Cibacron Blue F3GA–Zn(II)	Alkali Blue 6B–Zn(II)
0.5	92.7 ± 1.3	90.3 ± 2.7	92.9 ± 2.0
1.0	91.3 ± 2.0	91.6 ± 1.7	92.6 ± 1.5
2.0	94.6 ± 2.1	89.2 ± 2.0	91.2 ± 2.5
3.0	95.7 ± 1.7	91.6 ± 1.4	94.2 ± 2.4
4.0	92.3 ± 2.1	90.7 ± 2.2	96.5 ± 3.1
5.0	95.6 ± 2.0	93.5 ± 1.2	92.8 ± 2.5
6.0	94.9 ± 2.2	94.2 ± 1.5	92.2 ± 1.5
7.0	95.0 ± 2.4	92.7 ± 3.3	93.6 ± 2.0

Dye loading: 14.5 μmol/g; Zn(II) loading: 27.3 mg/g; BSA initial concentration: 5.0 mg/ml; pH: 6.0; and temperature: 20°C.

Zn(II) ions at this pH. These specific interactions may have resulted from the incorporated Zn(II) ions and BSA molecules as well as from the conformational state of BSA at this pH, as discussed in the related literature.[33,34]

Desorption and Repeated Use

The desorption of BSA from the dye/Zn(II)-derived microbeads was studied in an experimental batch setup. The microbeads, loaded with different amounts of BSA, were placed within the desorption medium containing 0.025*M* EDTA at pH 4.9, and the amount of BSA and Zn(II) desorbed in 1 h was determined. The desorption ratios for both BSA and Zn(II) were calculated by using the following expression:

$$\text{Desorption Ratio (\%)} = \frac{\text{amount of BSA desorbed [or Zn(II)]}}{\text{amount of BSA adsorbed [or Zn(II)]}} \times 100$$

Table I shows the desorption ratios of BSA for all dyes. A significant amount of the adsorbed BSA (around 95%) was easily desorbed from the dye/Zn(II)-derived poly(EGDMA–HEMA) microbeads in all cases when EDTA was used for desorption. Note that almost all of the Zn(II) ions initially loaded were desorbed from the microbeads. This means that EDTA breaks down the chelates between Zn(II) ions and dyes.

To show the reusability of the dye/Zn(II)-derived poly(EGDMA–HEMA) microbeads, the adsorption-desorption cycle was repeated three times using the same affinity sorbent (Fig. 11). The BSA adsorption capacities of the microbeads were observed to decrease to 4.3% for Congo red, 5.3% for alkali blue 6B, and 10.39% for Cibacron Blue F3GA, which were acceptable in all cases.

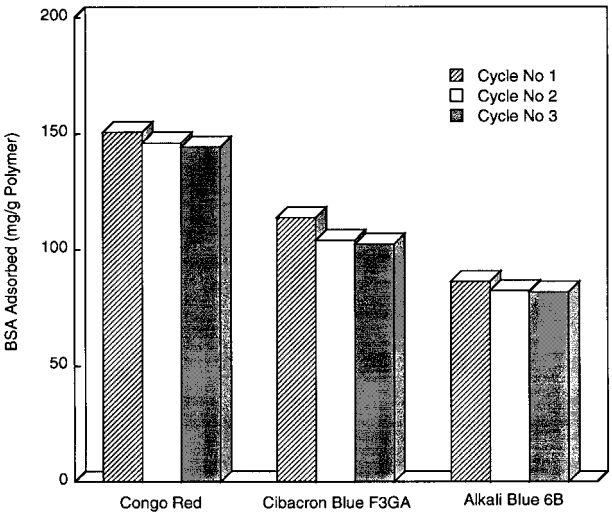

Figure 11 Repeated use of dye/Zn(II)-derived microbeads (dye loading: 14.5 μmol/g; Zn(II) loading: 27.3 mg/g; BSA initial concentration: 5.0 mg/ml; pH: 6.0; and temperature: 20°C).

CONCLUSIONS

1. High BSA adsorption capacities were obtained with dye-attached poly(EGDMA–HEMA) microbeads.
2. The highest BSA adsorption (90 mg BSA/g polymer) was observed with the Congo red-attached poly(EGDMA–HEMA) microbeads.
3. Incorporation of Zn(II) ions onto the dye-

attached microbeads further increased BSA adsorption capacities up to 159 mg BSA/g polymer.

4. It was possible to use of the dye/Zn(II)-derived microbeads in BSA adsorption-desorption cycles without noticeable loss of capacity.

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