Cibacron Blue F3GA Incorporated Magnetic Poly(2-hydroxyethyl methacrylate) Beads for Lysozyme Adsorption

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ABSTRACT: Lysozyme adsorption onto Cibacron Blue F3GA incorporated magnetic poly(2-hydroxyethyl methacrylate) (mag-PHEMA) beads was investigated. Mag-PHEMA beads (80–120 μm) were produced by dispersion polymerization. The triazine dye Cibacron Blue F3GA was incorporated covalently as a dye ligand. These dye-incorporated beads, having a swelling ratio of 34% (w/w) and carrying different amounts of Cibacron Blue F3GA (12.2–42.8 μmol/g) were used in lysozyme adsorption studies. The effects of the initial concentration, pH, ionic strength, and temperature on the adsorption efficiency of dye-incorporated beads were studied in a batch reactor. The effects of the Cibacron Blue F3GA loading on the lysozyme adsorption were also studied. The nonspecific adsorption of lysozyme on the mag-PHEMA beads was 0.8 mg/g. Cibacron Blue F3GA attachment significantly increased the lysozyme adsorption up to 295 mg/g. More than 90% of the adsorbed lysozyme was desorbed in 1 h in a desorption medium containing 1.0*M* KSCN at pH 8.0. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 719–725, 2004

Key words: adsorption; chromatography; dyes/pigments; magnetic polymers

INTRODUCTION

The rapid growth of biotechnology and biomedicine requires more reliable and efficient separation techniques for the isolation and purification of biomolecules such as proteins, peptides, nucleic acids, and hormones.[1] Techniques used on pilot scales for protein purification (e.g., electrophoresis, ultrafiltration, and bioaffinity chromatography) are excellent for producing small quantities of biomolecules. However, these processes are very difficult to scale up. Dye-affinity chromatography is an effective and widely used method for the purification of biomolecules.[2] However, it has a number of drawbacks, such as the compressibility of the column packaging materials (i.e., beads), fouling, and, in particular, the slow flow rate through columns.[3] To overcome these problems, the bead diameter has been reduced, but such carriers require high-pressure equipment.[4–6] The development of magnetic beads promises to solve many of the problems associated with chromatographic separations in packed-bed and conventional fluidized-bed systems.[7–10] Magnetic beads combine some of the best characteristics of fluidized beds (low-pressure drop and high feed-stream solid tolerances) and fixed beds (absence of particle mixing, high mass-transfer rates, and good fluid–solid contact).[11]

Lysozyme is a commercially valuable enzyme, and its common applications are (1) as a cell-disrupting agent for the extraction of bacterial intracellular products, (2) as an antibacterial agent in ophthalmologic preparations, (3) as a food additive in milk products, and (4) as a drug for the treatment of ulcers and infections.[12,13] The potential for its use as an anticancer drug has been demonstrated by animal and *in vitro* cell culture experiments.[14] Lysozyme has also been used in cancer chemotherapy.[15] In a recent article, it has been reported that lysozyme can be used to increase the production of immunoglobulin by hybridoma technology.[16]

In this study, a dye-affinity system using magnetic poly(2-hydroxyethyl methacrylate) (mag-PHEMA) beads as support matrices was prepared. A dye ligand, Cibacron Blue F3GA, was covalently incorporated into the mag-PHEMA beads, and the adsorption–desorption behavior of lysozyme was investigated. System parameters such as the bead properties (e.g., dye loading) and adsorption conditions (e.g., lysozyme concentration, pH, ionic strength, and temperature) were varied to evaluate their effects on the performances of dye-affinity beads.

EXPERIMENTAL

Materials

Lysozyme (chicken egg white, EC 3.2.1.7) was supplied by Sigma Chemical Co. (St Louis, MO) and used

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as received. 2-Hydroxyethyl methacrylate (HEMA) was obtained from Sigma Chemical and was distilled under reduced pressure in the presence of hydroquinone and stored at 4°C until it was needed. Ethylene glycol dimethacrylate (EGDMA; Merck, Darmstadt, Germany) was used as the crosslinking agent. Magnetite particles (Fe_3O_4; diameter < 5 μm) was obtained from Aldrich (Milwaukee, WI). *a,a′*-Azobisisobutyronitrile (AIBN) was supplied by Fluka AG (Buchs, Switzerland) and used as received. Cibacron Blue F3GA was obtained from Polyscience (Warrington, PA). All other chemicals were reagent-grade and were purchased from Merck. All water used in the adsorption experiments was purified with 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. The resulting purified water had a specific conductivity of 18 mS/cm.

Methods

Preparation of the mag-PHEMA beads

The mag-PHEMA beads were prepared by a dispersion polymerization technique in an aqueous medium as described in our previous article.[17] A typical suspension polymerization procedure for the mag-PHEMA beads was as follows. The dispersion medium was prepared by the dissolution of 200 mg of poly(vinyl alcohol) (molecular weight = 50.000) in 50 mL of distilled water. The desired amount of AIBN (60 mg) was dissolved in the monomer phase [12/4/8 EGDMA/HEMA/toluene (mL)] with 1 g of magnetite particles. This solution was then transferred into the dispersion medium placed in a magnetically stirred (at a constant stirring rate of 600 rpm) glass polymerization reactor (100 mL), which was in a thermostatic water bath. The reactor was flushed with bubbling nitrogen and then was sealed. The reactor temperature was kept at 65°C for 4 h. Then, the polymerization was completed at 90°C in 2 h. After the polymerization, the mag-PHEMA beads were separated from the polymerization medium. The residuals (e.g., unreacted monomer, initiator, and other ingredients) were removed by a cleaning procedure.

Dye attachment to the mag-PHEMA beads

Cibacron Blue F3GA was covalently incorporated into the mag-PHEMA beads via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl groups of poly(2-hydroxyethyl methacrylate) (PHEMA) under alkaline conditions. Various amounts of Cibacron Blue F3GA (0.25–2.0 mg/mL) were dissolved in 10 mL of water. This aqueous dye solution was transferred to mag-PHEMA beads in 90 mL of distilled water, and then 4 g of NaOH was added. The medium was heated at 80°C in a sealed reactor and was stirred magnetically for 4 h. The magnetic beads were washed several times with distilled water and methanol until all the physically adsorbed dye molecules were removed. Cibacron Blue F3GA incorporated mag-PHEMA beads were stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

The release of Cibacron Blue F3GA from the dye-incorporated beads was investigated at different pH values in the range of 4.0–8.0. These media were the same ones used in the lysozyme adsorption experiments described later. The Cibacron Blue F3GA release was also determined in a medium (pH 8.0) containing 1.0*M* KSCN, which was the medium used in the lysozyme desorption experiments. The medium with the Cibacron Blue F3GA incorporated magnetic beads was incubated at room temperature for 24 h. Then, the magnetic beads were removed from the medium, and the Cibacron Blue F3GA concentration in the supernatant was measured by spectrophotometry at 630 nm.

Characterization of the magnetic beads

Elemental analysis. The amounts of Cibacron Blue F3GA incorporated onto the mag-PHEMA beads were determined with a CHNS-932 elemental analysis instrument (Leco, Chicago, IL). The amount of Cibacron Blue F3GA incorporated onto the beads was calculated from these data on the basis of the sulfur stoichiometry.

Microscopic observations. Microscopic observations and photographs of the gold-coated magnetic beads were performed with a Leitz-AMR-1000 scanning electron microscope (Raster Electronen Microscopy, Leitz AMR-1000, Köln, Germany).

Porosity measurements. The pore volumes and average pore diameters greater than 20 Å were determined with a mercury porosimeter up to 2000 kg/cm^2 with a Carlo Erba (Milan, Italy) model 2000 instrument. The surface area of the magnetic beads was measured with a surface area apparatus [with the Brunauer–Emmett–Teller (BET) method].

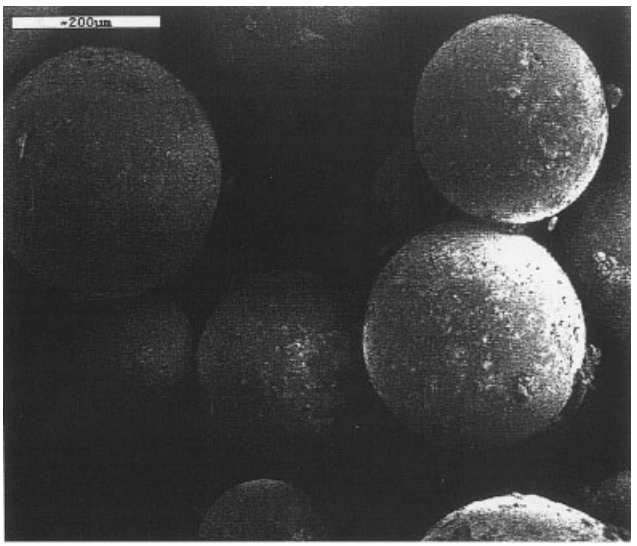
Adsorption–desorption studies with lysozyme

Lysozyme was selected as a model protein. Lysozyme adsorption of plain and Cibacron Blue F3GA incorporated mag-PHEMA beads was studied at various pHs. The pH of the adsorption medium was changed between 5.0 and 8.0 with different buffer systems (0.1*M* CH_3COONa–CH_3COOH for pH 5.0–6.0 and 0.1*M* Na_2HPO_4–KH_2PO_4 for pH 7.0–8.0). The initial concentration of lysozyme was changed between 0.1 and

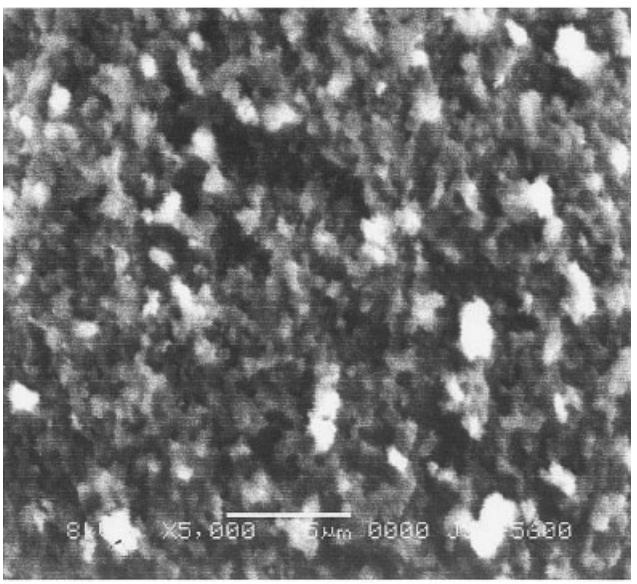
4.0 mg/mL. In a typical adsorption experiment, lysozyme was dissolved in 25 mL of a buffer solution, and beads (100 mg) were added. The adsorption experiments were carried out for 2 h at 20°C at a stirring rate of 100 rpm. The time to reach equilibrium adsorption with continuous stirring was found to be 2 h, and in the rest of the study, a 2-h adsorption duration was, therefore, employed. At the end of the equilibrium period, the beads were separated from the solution. The lysozyme adsorption capacity of the mag-PHEMA beads was determined through the spectrophotometric measurement (at 280 nm) of the concentration of lysozyme remaining in the adsorption medium. All the adsorption curves were averages of at least duplicated experiments.

The lysozyme desorption experiments were performed in a buffer solution containing 1.0*M* KSCN at pH 8.0. The lysozyme-adsorbed beads were placed in the desorption medium and stirred for 1 h at 25°C at a stirring rate of 100 rpm. The final lysozyme concentration within the desorption medium was determined by spectrophotometry. The desorption ratio was calculated from the amount of lysozyme adsorbed onto the beads and the amount of lysozyme desorbed.

To evaluate the effects of the adsorption conditions on the lysozyme structure, we recorded the fluorescence spectra of the native lysozyme, heat-denaturated lysozyme, and desorbed lysozyme. A native, aqueous lysozyme solution (1 mg/mL, pH 7.0) was denaturated at 70°C for 4 h. Fluorimetric measurements were taken with a Shimadzu spectrofluorometer (Tokyo, Japan) with 1-cm^2 quartz cells. Monochromatic readings were taken from a digital display with a 0.25-s time constant and with a 3-nm band width on the excitation side and a 5-nm band width on the emission side. The initial calibration was carried out with a standard solution of lysozyme in phosphate-buffered saline with a 280-nm fluorescence excitation wavelength and a 340-nm emission wavelength.



(a)



(b)

Figure 1 Scanning electron micrographs of mag-PHEMA beads: (a) general view (bar = 200 μm) and (b) surface (bar = 5 μm).

RESULTS AND DISCUSSION

Cibacron blue F3GA incorporated mag-PHEMA beads

Mag-PHEMA beads (80–120 μm) carrying Cibacron Blue F3GA were prepared as affinity adsorbents for lysozyme adsorption. Details of the preparation and characterization of mag-PHEMA beads can be found in our previous article.[17] The main selection criteria of mag-PHEMA are its mechanical strength and chemical stability. The mag-PHEMA beads prepared in this study were rather hydrophilic and crosslinked structures, that is, hydrogels. The simple incorporation of water weakened the secondary bonds within the hydrogels. This enlarged the distance between the polymer chains and caused the uptake of water. The equilibrium water uptake ratio of the mag-PHEMA beads was 34% (v/v).

The surface morphology and internal structure of the mag-PHEMA beads are exemplified by the electron micrographs in Figure 1. Figure 1(A) shows that the magnetic beads were spherical and had a rough surface because of the abrasion of magnetite crystals (diameter < 0.1 μm) during the polymerization procedure. Figure 1(B) was taken with broken beads so that we could observe the internal part. The presence of macropores within the bead interior can be clearly seen in this photograph. It can be concluded that the

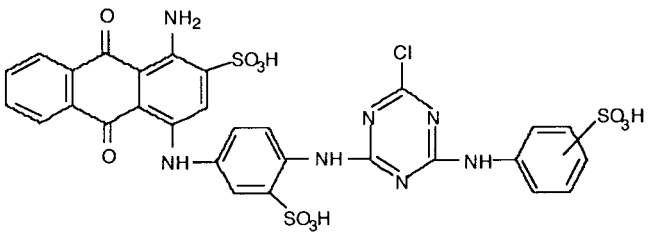


Figure 2 Chemical structure of Cibacron Blue F3GA.

mag-PHEMA 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 macropores reduced the diffusional resistance and facilitated mass transfer because of the microporous structure. This also provided higher Cibacron Blue F3GA incorporation and enhanced the lysozyme adsorption capacity.

According to the mercury porosimetry data, the pore radii of the mag-PHEMA beads changed between 175 and 400 nm. This indicated that the beads contained mainly macropores. This pore diameter range was possibly available for the diffusion of the lysozyme molecules. The molecular size of lysozyme was 4.0 nm × 4.0 nm × 1.9 nm. On the basis of these data, we concluded that the magnetic beads had effective pore structures for the liquid chromatography separation of lysozyme. The specific surface area of the magnetic beads was found to be 56 m^2/g by the BET method. After Cibacron Blue F3GA attachment, the specific surface area was found to be 56 m^2/g. Therefore, these pores were not blocked by the dye molecules.

The presence of magnetite particles in the polymeric structure was confirmed by electron spin resonance. The magnetic properties of the polymeric structure were also presented with electron mass units, which showed the behavior of the magnetic beads in a magnetic field with a vibrating magnetometer.

Cibacron Blue F3GA is a monochlorotriazine dye (Fig. 2), and it contains three sulfonic acid groups and four basic primary and secondary amino 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 surfaces that prefer to interact with hydrophobic residues in proteins. The Cibacron Blue F3GA molecules were covalently incorporated into the mag-PHEMA beads. It is a widely used dye ligand (even in 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. The Cibacron Blue F3GA incorporated magnetic beads were extensively washed to ensure that there was no dye leakage from any of the dye-incorporated beads and in any media used at adsorption or desorption steps.

Lysozyme adsorption–desorption studies

Effect of the cibacron blue F3GA loading

The effect of the Cibacron Blue F3GA loading on the lysozyme adsorption is shown in Figure 3. As expected, higher levels of lysozyme adsorption were observed in the more highly Cibacron Blue F3GA loaded mag-PHEMA beads. The lysozyme adsorption capacity increased with increasing Cibacron Blue F3GA loading in PHEMA beads up to 28.5 μmol/g. At higher Cibacron Blue F3GA loadings, however, the increases in the lysozyme adsorption capacity were slightly small. This decrease was probably due to steric effects such as Cibacron Blue F3GA coverage and pore blockage by adsorbed lysozyme molecules. The Cibacron Blue F3GA incorporated mag-PHEMA beads contained 42.8 μmol/g, which was the maximum value reached. The maximum lysozyme adsorption was 208.4 mg/g. Over the 28.5 μmol/g Cibacron Blue F3GA loading, steric hindrance between the dye and lysozyme molecules was important, and in this case, all the Cibacron Blue F3GA molecules could not be used for lysozyme attachment.

Effect of the initial concentration of lysozyme

Figure 4 shows the effect of the initial lysozyme concentration on the adsorption. As the lysozyme concentration increased in the solution, the amount per unit

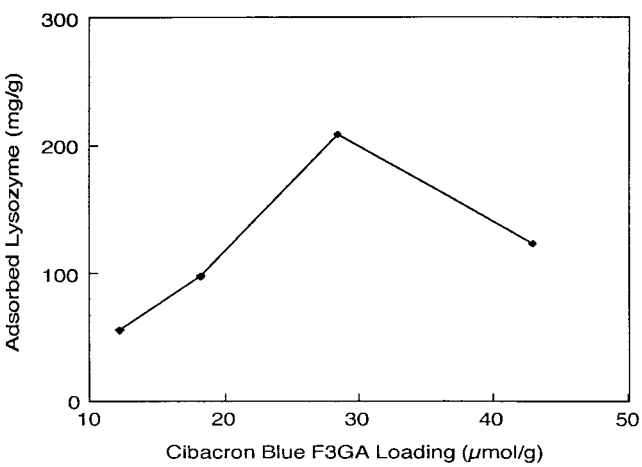


Figure 3 Effect of the Cibacron Blue F3GA loading on the lysozyme adsorption onto dye-incorporated mag-PHEMA beads (initial lysozyme concentration = 1.0 mg/mL; pH = 7.0; temperature = 25°C).

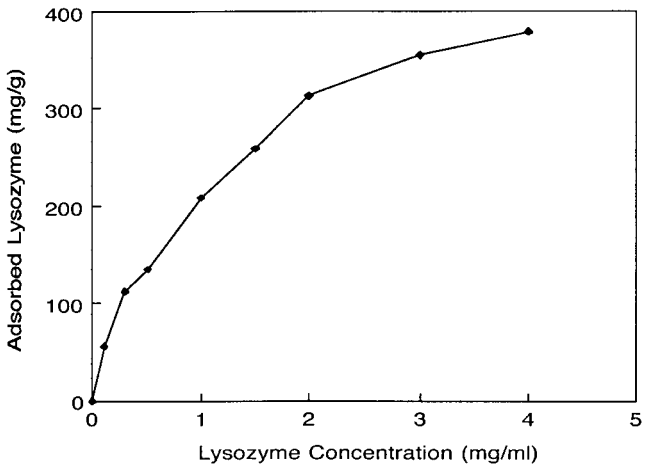


Figure 4 Effect of the lysozyme concentration on the lysozyme adsorption (Cibacron Blue F3GA loading = 28.5 μmol/g; pH = 7.0; temperature = 25°C).

of mass of lysozyme adsorbed by the beads increased and then approached saturation. The slope of the initial part of the adsorption curve represents a high affinity between lysozyme and Cibacron Blue F3GA molecules. It became constant when the protein concentration was greater than 3.0 mg/mL. A negligible amount of lysozyme adsorbed nonspecifically on the plain PHEMA beads (0.8 mg/g). Cibacron Blue F3GA attachment significantly increased the lysozyme adsorption capacity of the beads, up to 380 mg/g. It is clear that this increase was due to a specific interaction between Cibacron Blue F3GA and lysozyme molecules.

Effect of the pH

The amount of lysozyme adsorbed onto the Cibacron Blue F3GA incorporated mag-PHEMA beads as a function of the pH is shown in Figure 5. Lysozyme is highly positively charged at pH 7.0 (isoelectric point of lysozyme = 11.2). However, the amount of lysozyme adsorbed onto Cibacron Blue F3GA incorporated mag-PHEMA beads showed a maximum at pH 7.0, with a significant decrease at lower and higher pHs. Specific interactions (hydrophobic, electrostatic, and hydrogen-bonding) between lysozyme and dye molecules at pH 7.0 may have resulted from the ionization states of several groups on both Cibacron Blue F3GA (i.e., sulfonic acid and amino) and amino acid side chains in lysozyme and from the conformational state of lysozyme molecules (a more folded structure) at this pH. At pH values lower and higher than pH 7.0, the adsorbed amount of lysozyme drastically decreased. This could have been due to the ionization state of lysozyme and could have been caused by repulsive electrostatic forces between lysozyme and the dye molecules. An increase in the conformational size and the lateral electrostatic repulsions between adjacent adsorbed lysozyme molecules may also have caused a decrease in the adsorption efficiency.

Different polymeric sorbents for the affinity chromatography of lysozyme have been reported. Chen et al.[19] investigated the lysozyme adsorption capacity on a hydrophilic gel, and the maximum lysozyme adsorption capacity of the adsorbents was 84 mg/g. Horstman and Kenny[20] reported 15.1–16.6 mg/g lysozyme adsorption on Cibacron Blue F3GA/Sepharose CL-6B. Nash and Chase[21] modified a poly(styrene divinylbenzene) chromatography matrix through the adsorption and crosslinking of poly(vinyl alcohol), and they incorporated the dye ligand Procion Yellow HE-3G for human serum albumin and lysozyme adsorption. Their lysozyme adsorption efficiencies were 11.2–20 mg/mL. Champluvier and Kula[22] used nylon-based microfiltration membranes containing triazine dyes for lysozyme adsorption, and they reported an 8.6 mg/mL adsorption capacity. Nash et al.[23] investigated lysozyme adsorption onto Procion MX-R incorporated poly(styrene divinylbenzene) matrices, and they achieved a 68 mg/g adsorption capacity. A macroporous chitin membrane was used as an affinity membrane for lysozyme separation with high selectivity and high adsorption capacity at saturation (50 mg/mL membrane).[24] Chetty and Burns[25] investigated Macrosorb KAX CB for protein separation, and they reported a 23 mg/mL lysozyme adsorption capacity (the commercial dye-affinity resin Macrosorb KAX CB is a composite of silica and agarose onto which the ligand Cibacron Blue F3GA has been derivatized). Denizli et al.[26] prepared Congo Red carrying monosize poly(2-hydroxyethyl methacrylate methyl methacrylate) beads for protein separation, and they obtained a 67.6 mg/g lysozyme adsorption capacity. Sharma and Agarwal[27] investigated lysozyme adsorp-

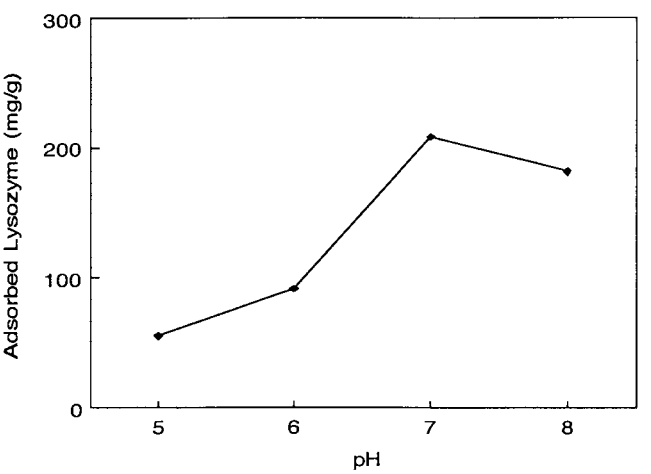


Figure 5 Effect of the pH on the lysozyme adsorption (lysozyme concentration = 1.0 mg/mL; Cibacron Blue F3GA loading = 28.5 μmol/g; temperature = 25°C).

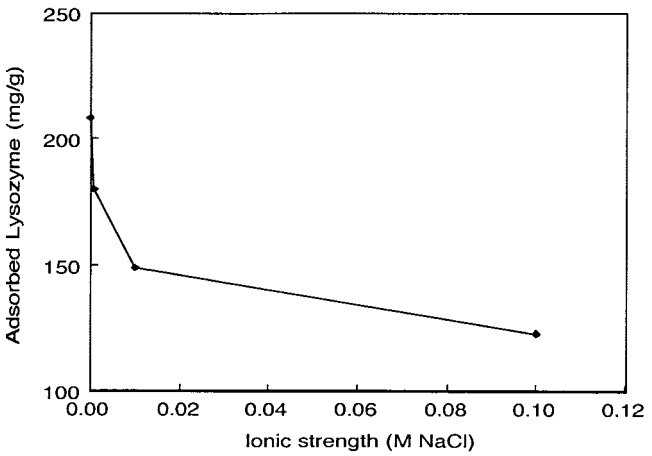


Figure 6 Effect of the ionic strength on the lysozyme adsorption (Cibacron Blue loading = 28.5 μmol/g; lysozyme concentration = 1 mg/mL; pH = 7.0; temperature = 25°C).

tion onto immobilized metal-affinity Sepharose 6B beads carrying different metal-complexing agents and metal ions, and they obtained an adsorption capacity of 6.8–190 mg/g. McCreath et al.[28] functionalized a poly(vinyl alcohol)-coated particulate perfluoropolymer with ion-exchange groups, and the equilibrium maximum adsorption capacity was 31.2 mg/mL. Garke et al.[29] studied the adsorption behavior of lysozyme on the strong cation exchanger Streamline SP, and they adsorbed 100 mg of lysozyme/g of polymer. The maximum lysozyme adsorption that we achieved in this study was 390 mg/g, which was quite high according to the related literature.

Effect of the ionic strength

The effect of the ionic strength (adjusted by the addition of NaCl) on the lysozyme adsorption is presented in Figure 6, which shows that the adsorption capacity decreased as the ionic strength of the binding buffer (acetate buffer, pH 7.0) increased. The adsorption of lysozyme decreased by about 40% as the NaCl concentration changed from 0.001 to 0.1*M*. The decrease in the adsorption capacity as the ionic strength increased can be attributed to the repulsive electrostatic interactions between the dye-incorporated magnetic beads and lysozyme molecules. When the salt concentration increased in the adsorption medium, this could lead to coordination of the deprotonated sulfonic acid groups of the dye with sodium ions of the salt (NaCl), and this could lead to low protein adsorption. The distortion of existing salt bridges in the presence of salt also contributed to this low protein adsorption at a high ionic strength.

Effect of the temperature

The effect of the temperature on the lysozyme adsorption capacity of the dye-affinity beads is presented in Figure 7. At all temperatures, the nonspecific adsorption of lysozyme was very low. No significant effect of the temperature was observed on the physical adsorption of lysozyme onto the plain beads. However, the equilibrium adsorption of lysozyme onto the Cibacron Blue F3GA incorporated magnetic beads significantly increased with increasing temperature. A possible explanation for this behavior is as follows. A chemical interaction between the dye and lysozyme molecules increased with increasing temperature.[30] The hydrophobic interactions were largely entropic in nature, whereas the electrostatic interactions contributed to the enthalpy.[31] The thermodynamic factor controlling the specific adsorption process, the overall free energy change associated with binding, probably had a rather complex temperature dependence because this term included both enthalpy and entropy changes.

Desorption

The desorption of the adsorbed lysozyme from the Cibacron Blue F3GA incorporated mag-PHEMA beads was studied in a batch experimental setup. Beads carrying different amounts of lysozyme were placed within a desorption medium containing 1.0*M* KSCN at pH 8.0, and the amount of lysozyme released in 60 min was determined. The desorption ratios for lysozyme were calculated with the following expression:

$$\text{Desorption ratio (\%)} = \frac{\text{Amount of lysozyme released}}{\text{Amount of lysozyme adsorbed}} \times 100$$

More than 95% of the adsorbed lysozyme was removed in all cases when KSCN was used for desorp-

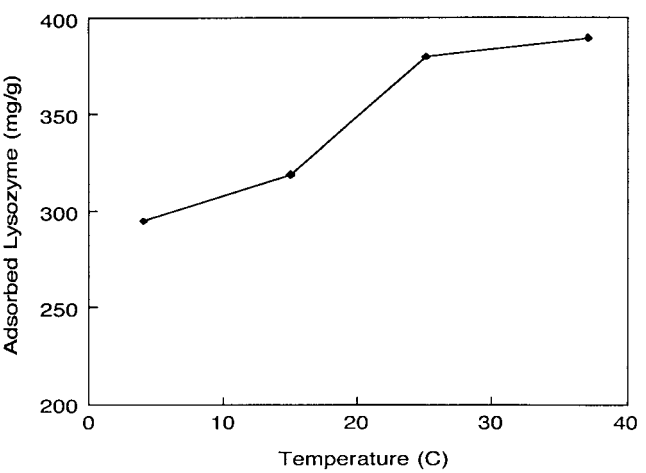


Figure 7 Effect of the temperature on the lysozyme adsorption (Cibacron Blue F3GA loading = 28.5 μmol/g; lysozyme concentration = 4.0 mg/mL; pH = 7.0).

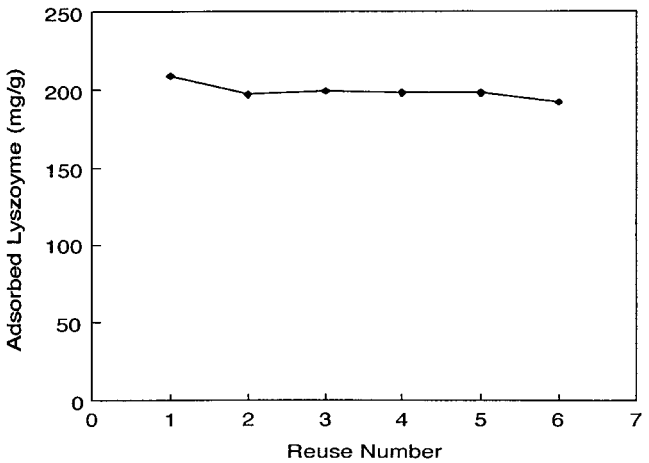


Figure 8 Repeated use of the beads (Cibacron Blue F3GA loading = 28.5 μmol/g; lysozyme concentration = 1.0 mg/mL; pH = 7.0; temperature = 25°C).

tion. From the lyotropic series, SCN— is a chaotropic anion, which can enhance protein elution.[32] This means that KSCN breaks down the bonds between lysozyme and Cibacron Blue F3GA molecules. With the aforementioned desorption data, we concluded that KSCN is a suitable desorption agent that allows the repeated use of the affinity sorbents developed in this study.

To show the reusability of the Cibacron Blue F3GA incorporated mag-PHEMA beads, we repeated adsorption–desorption cycle of lysozyme five times with the same beads. As shown in Figure 8, the adsorption capacities for the polymeric beads did not noticeably change during the repeated adsorption–desorption operations.

To evaluate the effects of the adsorption conditions on the lysozyme structure, we used fluorescence spectrophotometry. The fluorescence spectra of lysozyme samples obtained from the elution step were recorded. The fluorescence spectra of native and heat-denaturated lysozyme were also recorded. A clear difference was observed between the fluorescence spectra of native lysozyme and heat-denaturated lysozyme. An appreciable shift was seen in the maximum wavelength of denaturated lysozyme with respect to the native one. However, the fluorescence spectra of the samples withdrawn from the elution step were very close to those of native lysozyme, and no significant shift of the maximum wavelength was detected in the spectra of these samples in comparison with that of native lysozyme. It may be concluded that dye-affinity chromatography with mag-PHEMA beads can be applied to lysozyme separation without any conformational changes or denaturation.

CONCLUSIONS

Mag-PHEMA beads were produced by a dispersion polymerization technique with HEMA. Dye ligand Cibacron Blue F3GA was covalently incorporated onto the magnetic beads with a surface concentration of 12.2–42.8 μmol/g. Adsorption–desorption studies of lysozyme on Cibacron Blue F3GA incorporated mag-PHEMA beads led to the following conclusions. The lysozyme adsorption capacity of the dye-incorporated beads was 390 mg/g. The adsorbed lysozyme molecules were desorbed up to 97% with 1.0*M* KSCN as the desorption agent. To examine the effects of the adsorption conditions on the conformational changes of lysozyme molecules, we used fluorescence spectrophotometry. It appears that dye-affinity chromatography with modified magnetic beads can be applied to the adsorption of lysozyme without any denaturation. Repeated adsorption–desorption processes showed that these dye-incorporated magnetic beads are suitable for lysozyme adsorption.

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