Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 11 September 2000

To cite this Article Arica, M. Yakup and Denizli, Adil(2000) 'Performance of Different Metal-Dye Chelated Affinity Adsorbents of Poly(2-Hydroxyethyl Methacrylate) in Lysozyme Separation', Separation Science and Technology, 35: 14, 2243 — 2257
To link to this Article: DOI: 10.1081/SS-100102100
URL: http://dx.doi.org/10.1081/SS-100102100

SEPARATION SCIENCE AND TECHNOLOGY, **35**(14), pp. 2243–2257, 2000

Performance of Different Metal–Dye Chelated Affinity Adsorbents of Poly(2-Hydroxyethyl Methacrylate) in Lysozyme Separation

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ABSTRACT

The triazine dye Cibacron Blue F3GA was covalently immobilized as an affinity ligand onto microporous poly(2-hydroxyethyl methacrylate) (pHEMA) membranes. Three different metal ions [i.e., Fe(III), Zn(II), or Cu(II)] were then chelated with the immobilized Cibacron Blue F3GA molecules. Lysozyme adsorption onto these affinity adsorbents from aqueous solutions containing different amounts of lysozyme at different pH was investigated in a batch system. Lysozyme adsorption capacity of all of the metal-dye-immobilized membranes was greater than that of the dye-immobilized membranes. The nonspecific adsorption of the protein on the pHEMA membranes was negligible. The adsorption phenomena appeared to follow a typical Langmuir isotherm. The maximum capacity (q_m) of the Fe(III)–Zn(II),–or Cu(II)–dye chelated membranes for lysozyme adsorption (384, 326, and 306 $\mu g/cm^2$) was greater than that of the dye-immobilized membrane (224 μg lysozyme/cm^2), respectively. The dissociation constant (k_d) values were found to be 2.51×10^{-7} M with dye-immobilized membrane, and 2.32×10^{-7}, 2.38×10^{-7}, and 2.40×10^{-7} M with the Fe(III)–Zn(II),–and Cu(II)–dye-chelated membranes, respectively. More than 95% of the adsorbed lysozyme was desorbed in 60 min in the desorption medium containing 0.5 M KSCN at pH 8.0.

Key Words. Lysozyme; Microporous membrane; Cibacron Blue F3GA; Affinity adsorption; Metal chelate

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INTRODUCTION

Microporous membranes as support matrices for chromatography offer several potential advantages over traditional gel bead supports. Various pseudo dye ligands were immobilized onto different gel bead–based matrices, such as dextran (1), cellulose (2), agarose (3), polyacrylamide (4), chitin (5), and poly(vinyl alcohol) (6). However, their use is restricted by the compressibility of the soft gels at high flow rates, the high-pressure drops for small size beads, and the mass-transfer limitation resulting from the internal diffusion. A configuration in which the feed solution flows through the membrane allows the processing of a large amount of sample in a relatively short time owing to its structure, which provides a system with rapid reaction (7). The commercial membrane materials employed in affinity chromatography are limited to nylon, polyethersulfone, chitin, or cellulose (7–9). The membranes designed for affinity separation feature high microporosity, high chemical, biological, and mechanical stabilities, high degree of hydrophilicity, and the presence of functional groups, which permit attachment of a suitable ligand (10, 11). Poly(2-hydroxyethyl methacrylate) is a hydrogel that possesses high mechanical strength, resistance to many chemicals, and microbial degradation. These properties are very important in its use as a support material in affinity chromatography (12, 13). In addition, the hydroxyl groups of pHEMA offer protein attachment sites via activation and derivatization by introduction of a variety of ligands (14).

Triazine dyes are perhaps the most promising affinity ligands of large-scale potential and their corresponding adsorbents find wide applications in protein purification. Dyes offer advantages over biological ligands in term of economy, ease of immobilization, ligand stability, and adsorbent capacity (15–17). The only drawback of dyes appears to be their moderate selectivity for target proteins; this problem will be overcome by introducing new selectively interacting materials on the basis of their affinities for chelated metal ions. Such separations are generally based on the selective interaction between proteins containing one or preferably several adjacent histidine residues and chelated metal ions (18, 19). The number of histidine residues in the protein is of primary importance in the overall affinity with chelated metal ions. In addition, factors such as accessibility, microenvironment of the binding residue (i.e., histidine, cysteine, aspartic acid, glutamic acid, and tryrosine), cooperation between neighboring amino acid side chains and local conformations play important roles in biomolecule adsorption (20). Aromatic amino acids, free carboxylic groups, and the amino-terminus of the peptides also have some contributions (21–23). The low cost of metal ions and the ease of regeneration of the polymer-based adsorbents are the attractive features of metal affinity separation.

Lysozyme is found in a variety of vertebrate cells and secretions, such as spleen, milk, tears, and egg white. Lysozyme lyses certain bacteria by hydrolyzing the β-linkages between the muramic acid and *N*-acetylglucosamine of the mucopolysaccharides, which are present in the bacterial cell wall. It has many applications, e.g., in medicine as a bacteriolytic agent, wound therapeutic agent, and antibiotic activator (24, 25). The large-scale applications require efficient techniques for its isolation. Ratnayaka and Regnier (26) used a series of sorbents based on acrylate-grafted silica membranes and they reported 12.2–21.6 $\mu g/cm^2$ lysozyme immobilization capacity. Horstman and Kenny (27) reported that 15.1–16.6 mg/g lysozyme adsorbed on the Cibacron Blue F3GA immobilized onto Sepharose CL-6B. Champluvier and Kula (16) used microfiltration membranes as pseudoaffinity adsorbents and they showed 78–122 $\mu g/cm^2$ with the ultipor membrane containing different triazine dyes. Chen et al. (25) investigated lysozyme adsorption capacity on the hydrophilic gel; the maximum lysozyme adsorption capacity of the adsorbent was 6 μmol/mL gel (or 84 mg/mL gel). Nash and Chase (28) report that the lysozyme adsorption capacities of the modified poly(styrene-diviniylbenzene) and Procion Yellow HE-3G attached poly(vinyl alcohol) were in the range of 0.01–0.02 $\mu g/cm^2$.

In this study, microporous pHEMA membranes as the matrices have been prepared by UV-initiated photopolymerization. Dye–ligand (Cibacron Blue F3GA) was immobilized on a microporous membrane and was used as a metal–ligand chelate. Three different metal ions Fe(III), Zn(II), or Cu(II) were chelated on the dye–ligand, and the resulting affinity surfaces were then tested for their propensity to adsorb lysozyme in a batch system. The adsorption conditions (i.e., initial concentration of protein and medium pH) and the adsorption–desorption behavior of lysozyme were investigated.

EXPERIMENTAL

Materials

Lysozyme (chicken egg white, EC 3.2.1.7) was supplied from Sigma Chemical Co. (St. Louis, MO, USA) and used as received; 2-Hydroxyethyl methacrylate (HEMA) was obtained from Fluka AG (Switzerland), distilled under reduced pressure in the presence of hydroquinone and stored at 4°C until use; α-α′-Azoisobisbutyronitrile (AIBN) and Cibacron Blue F3GA were obtained from Sigma Chemical Co. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany).

Preparation of pHEMA Membranes

The membrane preparation mixture (5 mL) contained 2 mL HEMA, 5 mg AIBN as polymerization initiator, and 3 mL phosphate buffer (0.1 M, pH 7.0).

The mixture was then poured into a round glass mold (diameter: 9.0 cm) and exposed to ultraviolet radiation for 10 min, while a nitrogen atmosphere was maintained in the mold. The membrane was washed several times with distilled water and cut into circular pieces (diameter: 1.0 cm) with a perforator. The membrane thickness was 0.06 cm.

Cibacron Blue F3GA Immobilization onto pHEMA Membranes

Cibacron Blue F3GA (300 mg) was dissolved in distilled water (100 mL), and preequilibrated pHEMA membranes (15 g) were transferred in this dye solution. Sodium hydroxide (4 g) was then added to the medium and heated at 80°C for 4 h in a sealed reactor. After the reaction, the solution cooled to room temperature, then the Cibacron Blue F3GA-immobilized membranes were removed and washed thoroughly, first with distilled water and then with methanol, until all the physically bound dye was removed. The dye-immobilized membranes were stored at 4°C until use.

The amount of covalently immobilized Cibacron Blue F3GA on the membranes was evaluated by using an elemental analysis instrument (Leco, CHNS-932, USA), by considering the nitrogen and sulfur stoichiometry. The dye leakages from the affinity adsorbents were investigated in lysozyme adsorption and desorption media. The solution containing the affinity adsorbent was stirred for 24 h at room temperature. The concentration of leaked dye in the solutions was measured spectrophotometrically at 630 nm.

Chelation of Metal Ions on Dye-Immobilized Membranes

The dye-immobilized membranes were transferred to a buffer solution (pH 4.1, 0.1 M) containing 100 ppm Fe(III), Zn(II), or Cu(II) ions at 25°C while the solution was stirred magnetically for 60 min. $FeCl_3$, $Zn(NO_3)_2$, and $Cu(NO_3)_2$ were used as the source of Fe(III), Zn(II), and Cu(II) ions, respectively. The concentration of the each metal ion in the resulting solution was determined with a graphite furnace atomic absorption spectrophotometer (AAS, GBC 932 AA, Australia). Each metal ion leakage from the metal–dye chelated membranes was investigated in lysozyme adsorption (pH range 4.0–8.0) and desorption media (0.5 M KSCN at pH 8.0). Each metal–dye chelated membrane was stirred in these media for 24 h at room temperature. After this period, the leached Fe(III), Zn(II), or Cu(II) ions were determined in these solutions by using AAS.

Effect of pH on Lysozyme Adsorption

Lysozyme adsorption of the dye-immobilized and different metal–dye chelated membranes was studied at various pH levels, in either acetate (25

mL, 0.1 M, pH 4.0–5.5) or in phosphate buffer (25 mL, 0.1 M, pH 6.0–8.0). The initial lysozyme concentration was 500 μg/mL in the corresponding buffer. Each adsorption experiment was conducted for 120 min at 25°C in continuously stirring solution. After this period, the affinity adsorbents were removed from the lysozyme solution. The amount of adsorbed lysozyme on the affinity adsorbents was determined by measuring the initial and final concentrations of lysozyme within the adsorption medium by spectrophotometry at 280 nm. The assays were calibrated for lysozyme by measuring the optical density of lysozyme solutions of known concentration. All the adsorption curves are averages of duplicated experiments.

Adsorption Kinetics

To determine the adsorption capacities of the affinity adsorbents, initial concentration of lysozyme changed between 50–500 μg/mL in the adsorption medium. In a typical adsorption experiment, lysozyme was dissolved in phosphate buffer (25 mL, 0.1 M, pH 7.0) and the affinity adsorbents were added. The adsorption experiments were carried out for 120 min at 25°C at a stirring rate of 100 rpm. The time to reach equilibrium adsorption with continuous stirring was found to be 60 min, and in the rest of the study a 120-min adsorption duration was therefore employed. After the equilibrium period (i.e., 120 min), the affinity adsorbents were separated from the solution and the amount of adsorbed lysozyme was obtained by using

$$Q = [(C_o - C)V]/S \quad (1)$$

where Q is the amount of lysozyme adsorbed onto affinity adsorbents ($\mu g/cm^2$); C_o and C are the concentrations of the lysozyme in the initial solution and in the aqueous phase after adsorption, respectively (μg/mL); V is the volume of the aqueous solution (mL); S is the surface area of the affinity adsorbents in the adsorption medium (cm^2).

Stability of Affinity Adsorbents in Repeated Use

To determine the reusability of the affinity adsorbents, the adsorption and desorption cycle was repeated eight times by using the same affinity adsorbents. The lysozyme desorption experiments were performed in a buffer solution containing 0.5 M KSCN at pH 8.0 or 25 mM EDTA at pH 4.9. The lysozyme-adsorbed affinity adsorbents were placed in the desorption medium while stirring 100 rpm at 25°C for 60 min. The final lysozyme concentration in the desorption medium was determined by spectrophotometry. In the case of different metal–dye chelated membranes, desorption of Fe(III), Zn(II), and Cu(II) ions was also measured in the desorption media by means of AAS. The desorption ratios of lysozyme and each metal ion were calculated by using the

following expression:

Desorption ratio = [amount of lysozyme
× (or metal ions released) × 100] : [amount of lysozyme
× (or metal ions) adsorbed on the membranes]. (2)

RESULTS AND DISCUSSION

Microporous Affinity Adsorbents

Poly(2-hydroxyethyl methacrylate) membranes were prepared by UV-initiated photopolymerization of HEMA in the presence of an initiator (α-α′-azobisisobutyronitrile). An affinity dye (Cibacron Blue F3GA) was covalently immobilized on the membrane and different metal ions were chelated with the immobilized dye. It is reported that Cibacron Blue F3GA has no adverse effect on biochemical systems (29, 30). Cibacron Blue F3GA is a monochlorotriazine dye and it contains three sulphonic acid groups and four primary and secondary amino groups (Fig. 1). The strong binding of the Cibacron Blue F3GA to proteins occurs largely at the binding sites for substrates, coenzymes, and other prosthetic groups.

Metal recognition as a basis for protein separation in chromatography was introduced by Porath et al. in 1975 (21). Many studies have used divalent metal ions such as Cu(II), Ni(II), and Zn(II) and exploited the affinities for bases such as histidine via metal–ion coordination. The trivalent metal ions such as Fe(III) are considered hard Lewis acids and interact with hard Lewis bases such as oxygen. Molecules that have hard bases such as phosphates, carboxylates, sulphates, and phenolic groups have high binding constants. Softer Lewis acids such as divalent metal [i.e., Cu(II) and Zn(II)] ions prefer softer

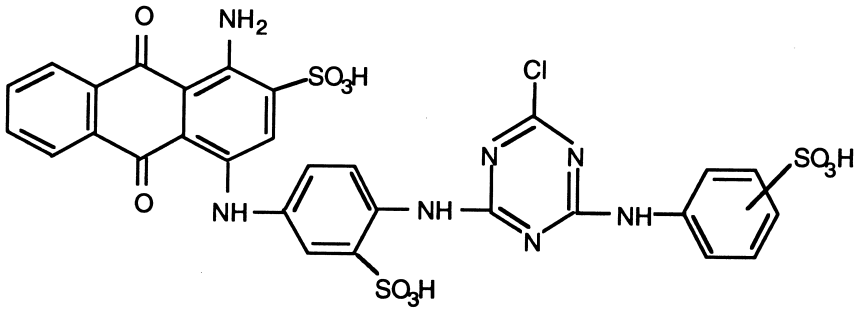

FIG. 1 Chemical structure of Cibacron Blue F3GA.

atoms such as nitrogen and sulfur as in cysteine and histidine. These are also electron donors to Fe(III) but form weaker complexes than those of the divalent metal ions (30–33).

Unmodified and dye-immobilized membranes were subjected to elemental analysis. The amount of dye on the membrane was calculated from this data (by considering the stoichiometry) to be 1.07 μM dye per cm^2. Studies aimed at detecting leakage of Cibacron Blue and metal ions [i.e., Fe(III), Zn(II), and Cu(II)] from the dye-immobilized and metal–dye chelated membranes revealed no leakage in any of the adsorption and desorption media, and implied that the washing procedure was satisfactory for the removal of the physically adsorbed Cibacron Blue F3GA molecules and metal ions from dye-immobilized membranes.

The adsorption equilibrium time of lysozyme on the affinity adsorbents was studied at pH 7.0 at 25°C (Fig. 2). Higher adsorption rates were observed during the initial adsorption process, then adsorption equilibria were achieved gradually in about 60 min. Adsorption of lysozyme molecules was very fast, especially when the lysozyme concentration was high. This behavior could be explained by concentration differences between the liquid and solid phases. As the initial concentration of lysozyme in the liquid phase increased, it caused a high driving force to the solid phases of lysozyme molecules. Lysozyme was adsorbed much faster onto the metals–dye chelated membranes than the dye-immobilized membrane because of the much higher affinity of the metals ions.

Effect of pH

The optimal pH values for adsorption of lysozyme on the affinity adsorbents were investigated in the pH range 4.0–8.0. As observed in Fig. 3, at pH 7.0, with [Fe(III), Zn(II), and/or Cu(II)]-dye-chelated membranes, 212, 185, and 165 μg/cm^2 lysozyme adsorptions were obtained, respectively, while with dye-immobilized membranes this was 133 μg/cm^2. Significantly lower lysozyme adsorption was obtained for all the adsorbents in acidic pH regions. These results indicate that the pH of the medium has an important effect on the adsorption equilibrium of lysozyme, and there is a preferential interaction between lysozyme and dye and/or different dye–metal chelated [i.e., Fe(III), Zn(II), and Cu(II)] molecules at around pH 7.0. The isoelectric (pl) value of lysozyme is 11.0. The number of charged groups on the surface of a protein would vary with the pH of the medium. These nonspecific interactions between lysozyme and affinity adsorbents may result from the cooperative effect of different mechanisms, such as hydrophobic interactions or ion-exchange effects, caused by the several aromatic structures, basic amino groups, and sulphonic acid groups on the Cibacron Blue F3GA and the amino acid sidechains of the surface of the lysozyme molecules.

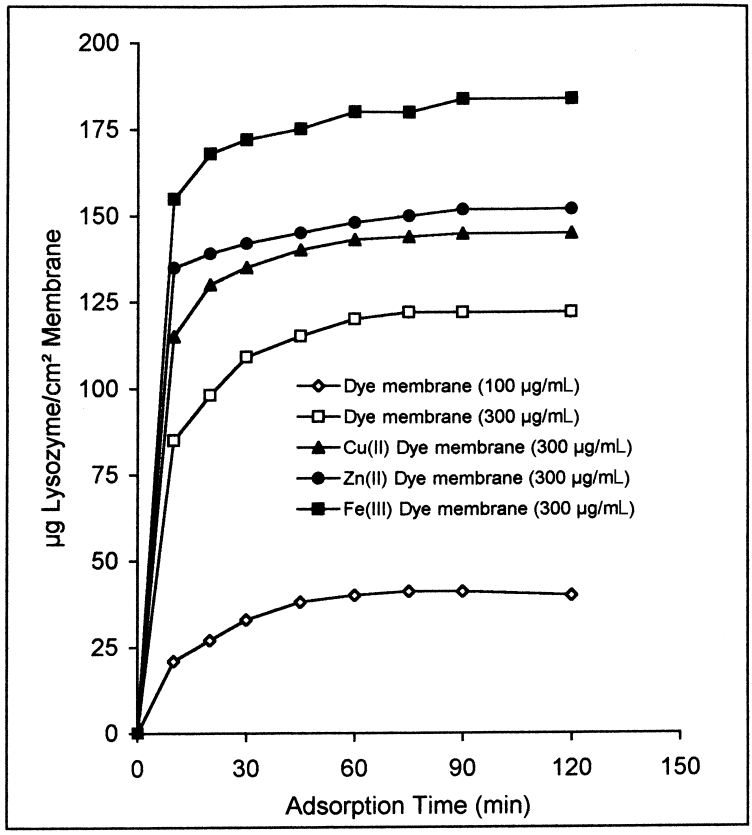

FIG. 2 Adsorption equilibrium time of lysozyme on the affinity adsorbents; pH: 7.0, temperature: 25°C.

Adsorption Isotherms

To compare the equilibrium adsorption curves of the same ligand-immobilized and metal-ions chelated affinity adsorbents, a series of adsorption experiments was carried out with various concentrations of lysozyme at pH 7.0 and 25°C. The equilibrium adsorption curves for the binding of lysozyme to Cibacron Blue F3GA immobilized and/or Fe(III), Zn(II), and Cu(II) ions chelated onto pHEMA membrane, are shown in Fig. 4. The loading of Fe(III), Zn(II), and/or Cu(II) ions onto the dye-immobilized membranes was 0.98, 2.35, and 2.11 $\mu M/cm^2$ membrane, respectively. Note that the dye-immobi-

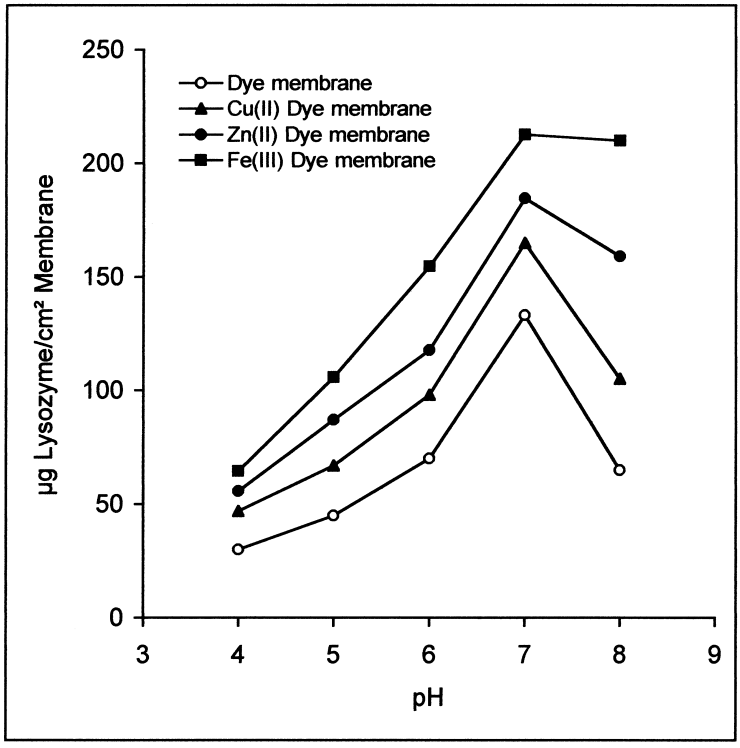

FIG. 3 Effects of pH on lysozyme adsorption on the affinity adsorbents. Initial concentration of lysozyme: 400 mg mL^{-1}, temperature: 25°C, time: 120 min.

lized membranes containing 1.07 μM Cibacron Blue F3GA per cm^2 membrane and the binding ratio of each metal ion to immobilized dye molecule was approximately two for divalent metal ions [i.e., Zn(II) and Cu(II)] and one for trivalent metal ions [Fe(III)]. A negligible amount of lysozyme (0.9 $\mu g/cm^2$) on the pHEMA membrane was nonspecifically adsorbed. Dye-immobilization increased the lysozyme adsorption capacity of the membrane 150-fold (133 $\mu g/cm^2$). As seen in Fig. 4, chelation of metal ions onto a dye-immobilized membrane leads to a further increase in the adsorption capacity of the affinity adsorbents to lysozyme. These were 1.6,- 1.4,- and 1.2-fold higher for Fe(III), Zn(II), and/or Cu(II) metal–dye-chelated membranes than for the dye-immobilized membrane, respectively. Another important observation was that an increase in lysozyme concentration in the adsorption medium led to an increase of the adsorbed lysozyme on the affinity adsorbents. This

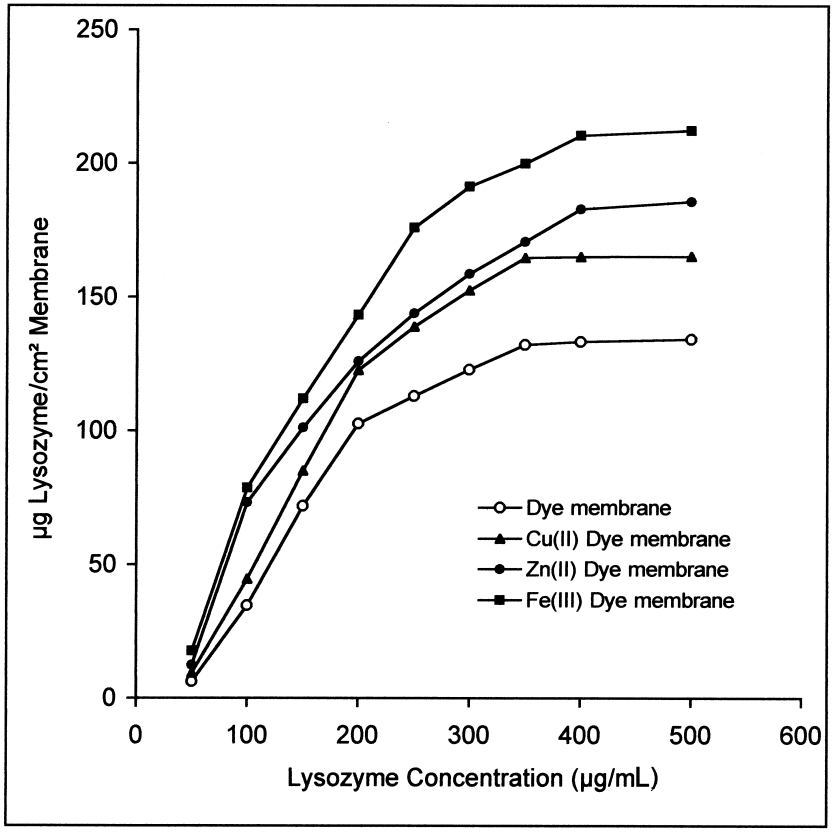

FIG. 4 Effect of lysozyme initial concentration on lysozyme adsorption on the affinity adsorbents; pH: 7.0, temperature: 25°C, time: 120 min.

was linear up to 200 μg lysozyme/mL, and it became constant when the lysozyme concentration was > 400 μg/mL. There was, thus, a maximum adsorption capacity level for the adsorbents. This could be considered a typical example of the occupation of all binding sites on the adsorbent surface that are available for lysozyme adsorption. In the affinity systems, more than one type of interaction mechanism is operational, such as the number of electron-donating groups on the protein surface, medium pH, concentration of protein, type of metal ions, ligand density, and type and size of chelating ligand (34).

It is necessary to determine which theoretical isotherm best fits the data. After the value of C^* (concentration of protein in solution) and q^* (amount of

protein adsorbed on the matrix) were obtained from experimental data, the semireciprocal plot of C^*/q^* versus C^* was employed (Fig. 5). The semireciprocal transformation of the equilibrium data gave rise to a linear plot for all the adsorbents and the respective correlation coefficient (R) was 0.998 for dye-immobilized and 0.995, 0.985, and 0.999 for Fe(III), Zn(II), and Cu(II) metal–dye chelated membranes, respectively, indicating that the Langmuir model could be applied in these systems. The model is described by

$$P + L \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PL \tag{3}$$

Protein and Ligand are donated by P and L, respectively. Where k_1 and k_2 are the forward and reversed interaction rates, respectively, which include the protein movement from the bulk phase to the adsorbent surface layer. Equation (3) can be expressed in the form of a rate equation with second-order for-

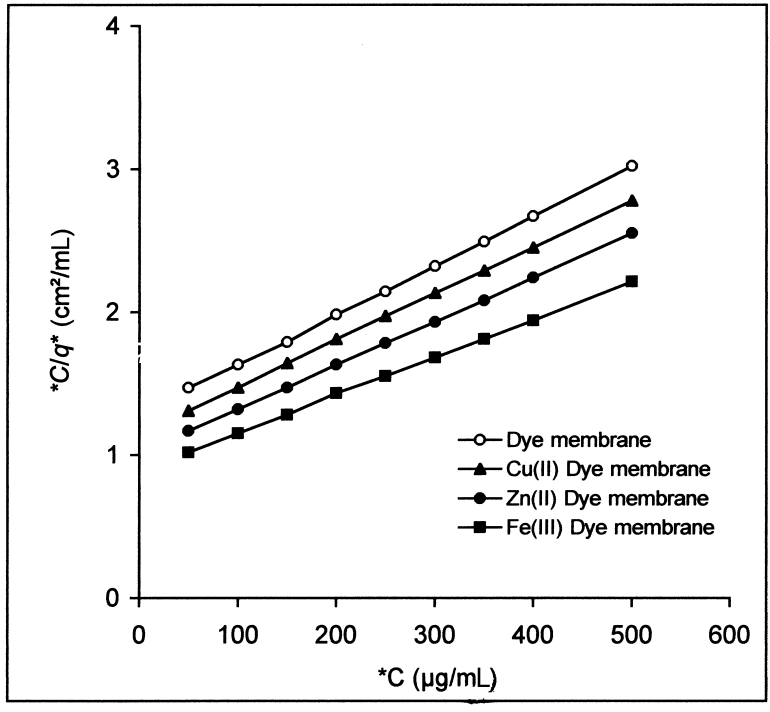

FIG. 5 Adsorption isotherm of lysozyme with dye-immobilized and [Fe(III), Zn(II), or Cu(II)] metal–dye-chelated membranes: pH: 7.0, temperature: 25°C, time: 120 min.

ward and first-order reverse kinetics, namely

$$dq/dt = k_1\, C^* \,(q_m - q^*) - k_2\, q \tag{4}$$

Where q_m is the maximum adsorption capacity of the matrix. At equilibrium, denoted by asterisk, the rate of forward interaction becomes equal to the rate of reverse interaction; dq/dt becomes zero and Eq. (4) can be converted to

$$q^* = q_m\, C^*/(k_d + C^*) \tag{5}$$

where $k_d = k_2/k_1$ is the dissociation constant of the system.

From the slopes, the maximum capacities (q_m) were found to be 224, 384, 326, and 306 μg/cm^2 for lysozyme when dye-immobilized, [Fe(III), Zn(II), and/or Cu(II)]–dye chelated membranes, respectively were used in the adsorption tests. Chelation of Fe(III), Zn(II), or Cu(II) ions onto the dye-immobilized membranes lead to significant increase in the q_m values of about 71%, 45%, and 36% of membranes to lysozyme, respectively. It is clear that this increase is due to ternary complex formation between Cibacron Blue F3GA, metal ions, and lysozyme molecules (i.e., metal ions promote the adsorption of lysozyme).

The distribution of hydrophobic and hydrophilic residues on the protein surface is a very important factor in complex formation with immobilized ligand on the polymer surface. In an aqueous medium, polar or charged residues tend to be on the surface and nonpolar residues in the interior. However, each amino acid residue cannot be distributed independently, owing to the primary structure of protein; the distribution varies from one protein to another. Thus, the hydrophobic amino acid residues are by no means all buried.

The concept of hardness–softness of metal ions may serve as a guide for understanding the principles and mechanisms of metal-affinity chromatography. According to these principles, Fe(III) ions have a strong affinity for hard bases such as phosphate, sulfate, and carboxylic and phenolic oxygen. The presence of four aspartic acid, two glutamic acid, and two tyrosine residues on the surface of lysozyme molecules provides an affinity for Fe(III) ions on the dye ligand through carboxylate and phenolic functional groups (35). The binding of proteins to soft metals [in this case Zn(II) and Cu(II)] occurs via the electron-donating side chains of residues such as histidine and cysteine. Lysozyme has only one histidine residue but has not cysteine residue on the surface. This exposed histidine residue should be the dominant binding site in lysozyme adsorption with chelated Zn(II) and Cu(II) metal ions. Binding should be a combination of metal coordination and ion-exchange interactions. Thus, the number of binding sites on the protein (aspartic acid, glutamic acid, tyrosine, and histidine residues) and the ligand–polymer structure influence metal affinity protein precipitation (18, 33).

The apparent dissociation constant (k_d) estimated from the intercept is a measure of the stability of the complex formed between a protein and an ad-

sorbent under specified experimental conditions. For example, a large k_d value indicates that the protein has a low binding affinity for the adsorbent. The k_d values were found to be 2.51×10^{-7} M for the dye-immobilized membrane and 2.32×10^{-7}, 2.38×10^{-7}, and 2.40×10^{-7} M for the Fe(III)–, Zn(II)–, and Cu(II)–dye-chelated membranes, respectively. Although the apparent k_d value for the four adsorbents is of the same order of magnitude, the metal-ions chelation on the dye-immobilized membrane led to a small reduction in the k_d values. This might be due to the metals–dye complex leading to better affinity than that of the dye complex with lysozyme molecules. Fe(III)–dye-chelate adsorbent also gave a slightly lower k_d value than the other adsorbents, because Fe(III) ions possibly interact with more than one side of the lysozyme molecules (six carboxylate and two phenolic surface groups are present on the lysozyme). The similarity of the k_d values observed for the [Zn(II) and Cu(II)]–dye-chelated adsorbents at pH 7.0 indicate that the interaction between both soft metal ions and lysozyme molecules are through the same binding side (i.e., histidine residue). The results reveal that a similar effect is exerted by chelated Zn(II) and Cu(II) on the adsorption of lysozyme in comparison with Fe(III) metal ions. The maximum lysozyme adsorption capacities achieved with the adsorbents developed in this study were in the range of 224–384 $\mu g/cm^2$ membranes, quite comparable to results from related studies.

Adsorption–Desorption and Reusability of Adsorbents

The desorption of the adsorbed lysozyme from the dye-derived and metal–dye-chelated membranes was studied in a batch system. The lysozyme-loaded adsorbents were placed within the desorption medium containing 0.5 M KSCN at pH 8.0 or 25 mM EDTA at pH 4.9, and the amount of lysozyme and Fe(III), Zn(II), or Cu(II) released in 1 h was determined. The amount of the adsorbed lysozyme was desorbed up to 95% in all cases when KSCN was used as a desorption agent. There was no Fe(III), Zn(II), or Cu(II) release in this case, which shows that metals ions are chelated to Cibacron Blue F3GA molecules on the membrane surface by strong chelate formation. However, when EDTA was used for desorption, about 45% of lysozyme was removed from dye-immobilized membranes, perhaps because of a salting-out effect, whereas under the same desorption conditions about 100% of the lysozyme was desorbed from the metal–dye chelated membranes. In the latter case almost all the Fe(III), Zn(II), or Cu(II) ions initially loaded were released from the membranes. This means that EDTA breaks down the chelates between Fe(III), Zn(II), or Cu(II) ions and Cibacron Blue F3GA molecules. The desorption data given above suggests that KSCN is a suitable desorption agent, especially for the different metal–dye-chelated membranes, and allows repeated use of the affinity adsorbents developed in this study. To show the

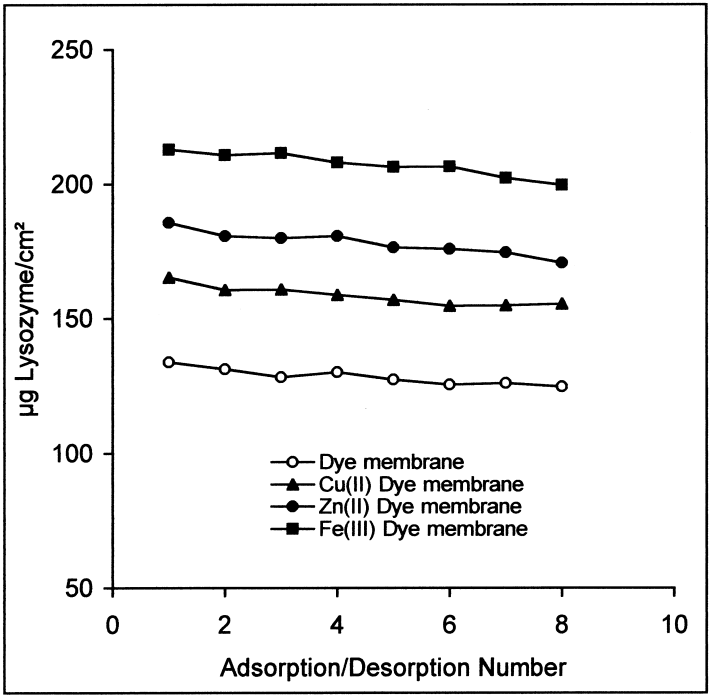

FIG. 6 Repeated use of dye-derived and dye–metal-chelated membranes. Initial concentration of lysozyme: 400 mg mL^{-1}, pH: 7.0, temperature: 25°C, time: 60 min. Desorption agents: 0.5 M KSCN at pH 8.0 or 25 mM EDTA at pH 4.9.

reusability of the dye-derived and different metal–dye-chelated membranes, the adsorption–desorption cycle of lysozyme was repeated eight times by using the same affinity adsorbents. As seen from Fig. 6, adsorption capacities for all the adsorbents did not noticeably change during the repeated adsorption–desorption operations.

CONCLUSION

The maximum capacity of the dye-immobilized membrane was 224 $\mu g/cm^2$. Chelation of Fe(III), Zn(II), or Cu(II) ions onto dye-immobilized membranes lead to significant increases in the q_m values, about 71%, 45%, and 36% of membranes to lysozyme, respectively. Adsorbed lysozyme molecules were desorbed up to 95% by using 0.5 M KSCN as the desorption agent. Repeated adsorption–desorption processes showed that these novel affinity adsorbents revealed good properties for adsorption of model adsorbate lysozyme

and will be effective in processing large volumes of culture medium containing a target protein.

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Received by editor August 5, 1999
Revision received December 1999