Preparation of Methacrylamide Grafted and Dye-Ligand Immobilized PET Fibers: Studies of Adsorption and Purification of Lysozyme

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ABSTRACT: In this study, novel affinity chromatographic fibers was prepared from methacrylamide grafted poly(ethylene terephthalate), PET-*g*-pMAA, using benzoylperoxide as an initiator. A dye ligand (i.e., Procion Brown) as a ligand was then covalently immobilized on the different amount of pMAAm grafted PET fibers, (PET-*g*-pMAAm-PB). The fibers were characterized by surface area measurement, infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). Adsorptive properties of the composite fibers were tested using a model protein (i.e., lysozyme). To achieve these purposes, the influence of pH, ionic strength, initial lysozyme concentration, and temperature on adsorption system has been investigated and evaluated. A maximum lysozyme adsorption PET-*g*-pMAAm-PB fiber was obtained as 43.9 mg g^{-1} at pH 7.5. The experimental equilibrium data obtained for lysozyme adsorption onto PET-*g*-pMAAm-PB fibers fitted well to the Langmuir isotherm model. The result of kinetic analyzed for lysozyme adsorption onto affinity fibers showed that the second-order rate equation was favorable. The purity of the eluted lysozyme, as determined by HPLC, was 84% with recovery 73% for PET-*g*-pMAAm-PB fiber. Experiments on regeneration and dynamic adsorption were also performed. It appears that PET-*g*-pMAAm-PB fibers can be applied for lysozyme separation without causing any denaturation. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 108: 3313–3323, 2008

Key words: PET fibers; methacrylamide; graft copolymerization; dye ligand; adsorption; lysozyme; isotherm model; kinetics

INTRODUCTION

Up to now, many different types of chromatographic support used in affinity chromatography have been developed for protein separation and purification such as agarose, crosslinked cellulose and dextran, and silica.[1–5] An ideal affinity matrix for chromatographic application should adsorb specifically a target biomolecule from biological fluids.[6,7] Synthetic polymeric materials are widely used in chromatographic areas because of different mechanical and chemical properties. These polymers containing hydrophilic surface functional groups, e.g., carboxyl, hydroxyl, amino or phosphate ones, which can create a net electrostatic potential, are often used for investigations of interactions between support and target biological molecules.[8–11] There are several reports that the amount of adsorbed biomolecules on a chromatographic support surfaces are quantitatively changed, depending on the type of functional groups present. The surface of the chromatographic support can be modified, such as grafting,[12] immobilization of triazidine dye,[13–15] amino acids,[16,17] and metal ions[18,19] etc., for selective interaction with biological molecules.

Poly(ethylene terephthalate) (PET) fibers are one of important synthetic fibers used in the textile industry. PET is resistant to acids, bases, some solvents, and oils and fats. It has some disadvantages such as high crystallinity, hydrophobicity, low moisture regain, and lack of chemically reactive groups. Great emphasis has been put on the modifications of PET fibers, such as graft copolymerization, to explore their full potential. Polymer characteristics can be improved by the addition of side chains onto PET fibers by graft copolymerization.[20,21] Acrylate monomers such as methacrylamide, methyl methacrylate, acrylic acid were grafted onto PET fiber using various techniques to improve their surface properties for creation of adsorptive surfaces.[20,22] In chromatographic applications, the modified polymers with hydrophilic monomer having low interfacial energies towards the biological surrounding are also important. Fiber-form alternatives to packed-bed polymer technologies have been investigated as

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stationary phases in HPLC for many of the reasons mentioned above along with assumed improvements in hydrodynamic characteristics and adsorptive properties.[23] In addition, the fibrous adsorbents have a very high adsorption capacity because of their very large surface area. Grafting of functional group-containing fibrous polymer chains onto fiber surface can also provide a large number of functional groups or modification sites for immobilization of affinity ligand.[24]

In this study, metharylamide was grafted on the PET fibers, and a dye-ligand was covalently immobilized on the amide groups of the grafted metharylamide brushes. The effects of reaction conditions on the graft polymerization, such as the initiator concentration, ratio of MAA to PET, temperature, and polymerization time were investigated and optimized in the previous study.[25] Metharylamide grafted fibers were characterized by FTIR spectroscopy, scanning electron microscopy, and thermogravimetric analysis. After graft copolymerization, a reactive dye (i.e., Procion Brown MX 5BR, (PB)) was immobilized onto the PET-*g*-pMAAm fibers. Lysozyme adsorption on the PET-*g*-pMAAm-PB fibers from aqueous solutions was investigated under different experimental conditions in a batch system. Adsorption equilibrium of lysozyme at different initial concentration was obtained in a series of batch experiments and the results were quantified in terms of the various isotherms and kinetic models.

EXPERIMENTAL

Materials

Poly(ethylene terephthalate) PET fibers (30 filaments, 110 dTex) were supplied from SASA (Adana, Turkey). Benzoylperoxide was obtained from Sigma Chemical Co. (St Louis, MO) and recyrstallized twice from a methanol/chloroform mixture and dried vacuum. Methacrylamide (MAAm) was obtained from Fluka AG (Switzerland) and recrystallized in benzene/methanol mixture. Lysozyme (chicken egg white, EC 3.2.1.7) and Procion Brown MX 5BR (Reactive Brown 10; MW 1163.58) were supplied from Sigma Chemical Co. (St Louis, MO) and used as received. All other chemicals were of analytical grade and were purchased from Merck AG (Darmstadt, Germany). The water used in the following experiments was purified using a Barnstead (Dubuque, IA) ROpure LP reverse osmosis system.

Preparation of methacrylamide grafted PET fibers

PET-*g*-pMAAm fibers containing various amount of MAAm were prepared by changing the concentration of monomer in the polymerization reaction as previously reported.[25] PET fibers prepared as bundles (0.3 ± 0.001 g) were washed with distilled water and Soxhlet-extracted for 6 h with acetone and dried at ambient conditions before graft copolymerization. The fibers were transferred into the reaction chamber containing various amount of MAAm monomer. The reaction chamber was then placed in a water bath and then heated up to 75°C. Polymerization was initiated by adding benzyl peroxide solution with the required concentration in 2 mL acetone. The graft polymerization reaction was carried out under nitrogen atmosphere for a predetermined period of time. At the end of this period, the MAAm grafted PET fibers were removed from the reaction chamber and washed with hot water and extracted in Soxhlet for 8 h with water to remove the homopolymer. The fibers were dried in vacuum at 50°C for 12 h.

The percentage graft yield was determined gravimetrically by the use of grafted and original weights of the PET fibers.

$$\text{Grafting percentage } \% = (W_2 - W_1/W_1) \times 100 \quad (1)$$

$$\text{Grafting efficiency } \% = (W_2 - W_1/W_3) \times 100 \quad (2)$$

where W_1, W_2, and W_3 denote the mass of original PET fibers, resultant MAAm grafted fibers and the corresponding sodium salt of the initially added MAAm in the reactive mixture, respectively.

Dye-ligand immobilization onto PET-*g*-pMAAm fibers

A dye ligand (i.e., Procion Brown MX 5BR) was covalently immobilized onto the pMAAm grafted PET fibers via the nucleophilic substitution reaction between the chloride of its triazine ring and the amide groups of the PET-*g*-pMAAm fibers under alkaline conditions (Fig. 1). Procion Brown MX 5BR (300 mg) was dissolved in distilled water (10 mL) and transferred to same medium (90 mL) in which different amount of p(MAAm) grafted fibers (1.5 g) were equilibrated. Sodium carbonate (10 g) was then added to the medium (about pH 12) and heated at 80°C for 4 h in a sealed reactor. After the reaction period, the solution cooled down to the room temperature and PET-*g*-pMAAm fibers were washed several times with distilled water, 2.0*M* NaCl, and 10% methanol. The dye-ligand immobilized PET-*g*-pMAAm fibers were dried in a vacuum oven at 30°C.

The amount of immobilized dye and the leakage of the immobilized dye from the PET-*g*-pMAAm-PR fibers was followed by incubating the fibers in 1.0*M* HCl solution for 6 h at 65°C and buffer solution (pH 8.0, 0.1*M*)) for one mount at room temperature, respectively. The released amounts of dye after these

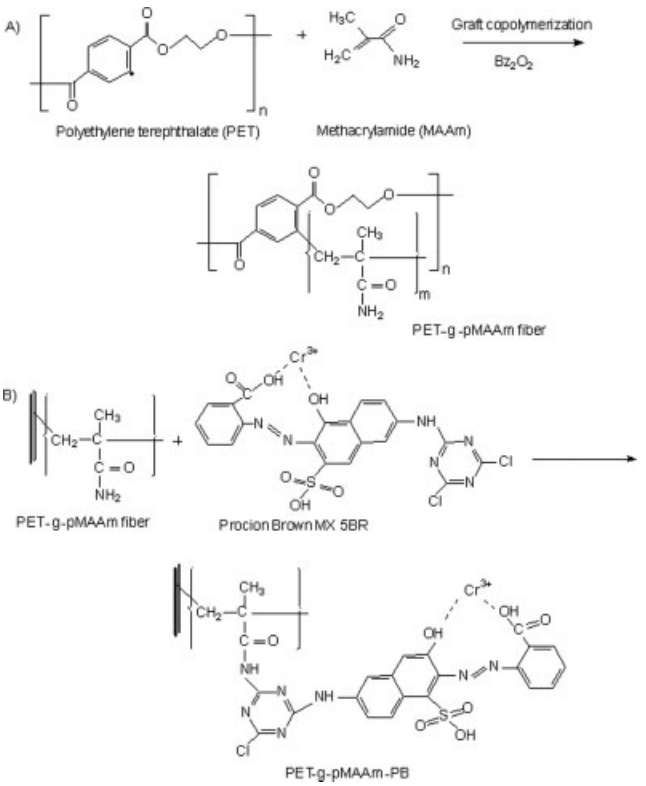

Figure 1 Schematic representation of the chemistry of the PET-*g*-pMAAm-PB fiber.

incubation periods were measured in the liquid phase at 574 nm using a UV–vis spectrophotometer.

Characterization studies

The surface morphology of PET and PET-*g*-pMAAm fibers was examined using scanning electron microscopy (SEM). Samples were coated with gold under reduced pressure, and the surface of the sample was then scanned at the desired magnification. FTIR spectra of PET, PET-*g*-pMAAm, PET-*g*-pMAAm-PB, and PET-*g*-pMAAm-PB-lysozyme fibers were obtained using an FTIR spectrophotometer (Shimadzu, FTIR 8000 Series, Japan). All fibers (0.1 g) and KBr (0.1 g) were thoroughly mixed and the mixture was pressed to form a tablet, and the spectrum was recorded. Thermogravimetric (TGA) analysis of PET, PET-*g*-pMAAm, PET-*g*-pMAAm-PB, and PET-*g*-pMAAm-PB-lyszyme fibers were obtained using a DSC-DTA instrument (Model DSC-60-DTG-60H, Schimadzu, Japan) at a heating rate of 10°C min^{-1} under nitrogen atmosphere. Samples were embedded in nonhermetic aluminum pans.

Adsorption of lysozyme from aqueous solution

The capacities of the fibers (i.e., PET, PET-*g*-pMAAm and PET-*g*-pMAAm-PB) were determined in a batch system. Adsorption of lysozyme from aqueous medium on the fibers was studied at different pHs, in either acetate (50 mM, pH 4.0–5.5) or in phosphate buffer (50 mM, pH 6.0–8.0). The initial concentration of lysozyme was 0.5 mg mL^{-1} in each corresponding buffer solution. The effect of grafting percentage (4.0–80.0%), ionic strength (0.0–1.0*M* NaCl), and temperature (4–37°C) on lysozyme adsorption were studied in phosphate buffer (50 mM, pH 7.5). To determine the adsorption capacity of the PET-*g*-pMAAm-PB fibers, the initial concentration of lysozyme was changed between 0.25 and 2.0 mg mL^{-1}. The amount of adsorbed lysozyme was determined by measuring the initial and final concentrations of lysozyme within the adsorption medium at 280 nm by using a double beam UV–vis spectrophotometer (Shimadzu, Tokyo, Japan, Model 1601). Calibration curves were prepared using lysozyme as standard (0.1–2.0 mg mL^{-1}). The amount of adsorbed protein was calculated as previously described.[14] Each experiment was performed in twice for quality control and statistical purposes.

The continuous system was made from Pyrex glass (length 6.0 cm, diameter 1.8 cm, total volume 15 mL). 1.5 g dry PET-*g*-pMAAm-PB fibers was soaked in deionized water for 24 h and then packed in a column (the bed volume, 13.6 mL). The column was equilibrated with phosphate buffer (0.1*M*, pH 7.5). Lysozyme solution (1.5 mg mL^{-1}) was prepared in phosphate buffer (50 mM, pH 7.5), and introduced into the column by means of peristaltic pump at a constant flow rate. When the adsorptive sides of the PET-*g*-pMAAm-PB fibers in the column were saturated, the column was washed with 50 mL of the same buffer solution to remove nonspecifically adsorbed lysozyme. Dynamic adsorption capacity (DAC) was calculated from breakthrough curves at 5% breakthrough point using the method reported previously by Griffith et al.[26] It is given as:

$$Q_{5\%} = u \times C_0 \int (1 - C)/C_0 dt \tag{3}$$

where $Q_{5\%}$ is the total amount of lysozyme adsorbed at 5% breakthrough point (mg g^{-1}), u is feeding rate (BV h^{-1}), t is the time of adsorption (h), C_0 and C are the concentrations of lysozyme in feeding solution and in effluent at the moment of t, respectively, (mg mL^{-1}). DAC is calculated as:

$$\mathrm{DAC} = Q_{5\%}/m \tag{4}$$

where m is the mass of adsorbent (g). Average retention time (τ) is calculated as:

$$\tau = \mathrm{BV}/u \tag{5}$$

where BV is bed volume of column (13.6 mL).

Desorption of lysozyme and reuse of affinity fibers

In all cases, bound lysozyme molecules onto the PET-*g*-pMAAm-PB fibers were desorbed by using 1.0*M* NaCl in phosphate buffer at pH 8.0 in batch experimental setup. Lysozyme adsorbed affinity fibers were placed in desorption medium and stirred continuously (at stringing rate 150 rpm) for 2.0 h at room temperature. The desorption ratio was calculated from the amount of lysozyme adsorbed on the beads and the final lysozyme concentration in the desorption medium. To show the reusability of the beads, the protein adsorption–desorption procedure was repeated five times by using the same PET-*g*-pMAAm-PB fibers. When desorption was achieved, the PET-*g*-pMAAm-PB fibers was regenerated with 50 mM NaOH solution and then reequilibrated with the same starting buffer.

To evaluate the effects of adsorption conditions on the conformational changes of the lysozyme, native, desorbed, and heat-denaturated lysozyme were measured with fluorescence spectrometer (Model RF-5301 PC, Shimadzu Japan). Native lysozyme solution (1 mg mL^{-1}, pH 7.5) was denaturated at 70°C for 2 h. The fluorescence spectrum of lysozyme was measured from 220 to 390 nm using a fluorescence spectrometer. The excitation wavelength was set at 295 nm.

Purification of lysozyme from egg white

Chicken egg white was separated from fresh eggs and diluted to 50% (v/v) with phosphate buffer (50 mM, pH 8.0). The diluted egg-white was homogenized in an ice bath and centrifuged at 4°C, at 10,000 rpm for 30 min. The supernatant fluid was then used as a lysozyme source. PET-*g*-pMAAm-PB affinity fibers (200 mg) were incubated with 20 mL of diluted egg-white solution for 2 h, in flasks agitated magnetically at 100 rpm. After this period, the PET-*g*-pMAAm-PB fibers were washed to remove nonspecifically bound proteins and the adsorbed lysozyme was desorbed as described earlier.

In the purification experiments, the activity of lysozyme was determined spectrophotometrically at 620 nm as previously described, the decrease in the turbidity of culture of *Micrococcus lysodeikticus* cells suspended in phosphate buffer (0.1*M*, pH 7.0) was followed for 5 min after addition of lysozyme. One unit lysozyme activity was defined as the amount of enzyme causing a decrease of 0.001 optical density values per minute at 25°C and pH 7.0. To examine the purity of the lysozyme in the purified samples, was used a Dionex HPLC (Dionex Co., Germering, Germany) system.

The HPLC system consisted of a quaternary pump with an on-line vacuum degasser (Model P580 A), a column oven (Model STH 585), an UV-visible diode array detector (Model 340 S), and an autosampler with a variable injection capacity from 1 to 250 μL (Model ASI-100). Chromatographic separation of proteins was achieved on a Discovery BIO Wide Pore C5 (15 cm × 4.6 mm i.d, sample particle size 5 μm) column and protected by a guard column (25 cm × 4.6 mm i.d). HPLC mobile phase A and B were prepared by adding trifluoroacetic acid (TFA, 0.1%, v/v): in Milli Q water (75%): acetonitril (25%) and acetonitril (25%): Milli Q water (75%), respectively. The chromatographic separation was performed using a gradient at 1.0 mL min^{-1} flow-rate (0–25 min, phase B from 0 to 100%) and the sample injection volume of the autosampler was 20 μL. The UV–vis detector was set at 220 nm and the temperature was maintained at 25°C. Dionex CHROMELEON® software was used and operated under Windows 98 for data acquisition and integration.

RESULTS AND DISCUSSION

In this study, poly(ethylene terephthalate) (PET) was selected as a base polymer for its chemical functionality and stability over a wide range of pH (1–14). A two-step process was carried out for the preparation of affinity fibers. In the first step, methacrylamide grafted PET fibers was prepared by graft copolymerization of MAAm onto PET fibers in a homogenous system using benzoyl peroxide as an initiator (Fig. 1). The graft yield was observed to increase with temperature and monomer concentration. The highest graft yield was obtained with an initiator concentration of 1×10^{-2} mol L^{-1}. The rate of grafting was proportional to the power 0.48 and 1.60 of the methacrylamide and benzoyl peroxide concentrations, respectively.[25] In the second step, a dye ligand (i.e., Procion Brown MX5-BR) was covalently immobilized onto modified fibers (PET-*g*-pMAAm-PR) (Fig. 1). Thus, a large number of new functional-sites on the PET-*g*-pMAAm-PB fibers were created for interactions of various biomacromolecules. In fact, pMAAm and dye ligand were tethered on the fibers surface to prepare dual biomimetic chromatographic support for protein separation.

The scanning electron micrographs of bare PET fibers and grafted PET fibers (44.6%) are presented in Figure 2. The bare PET fibers surface [Fig. 2(a)] has a smooth and relatively homogeneous appearance. The SEM micrograph shows that the graft copolymer greatly improved the compatibility of the PET-*g*-pMAAm composite [Fig. 2(b)]. The grafted side chain, pMAAm, seems to form microphases attached to the PET back-bone in the graft copolymer which is further proof of grafting.

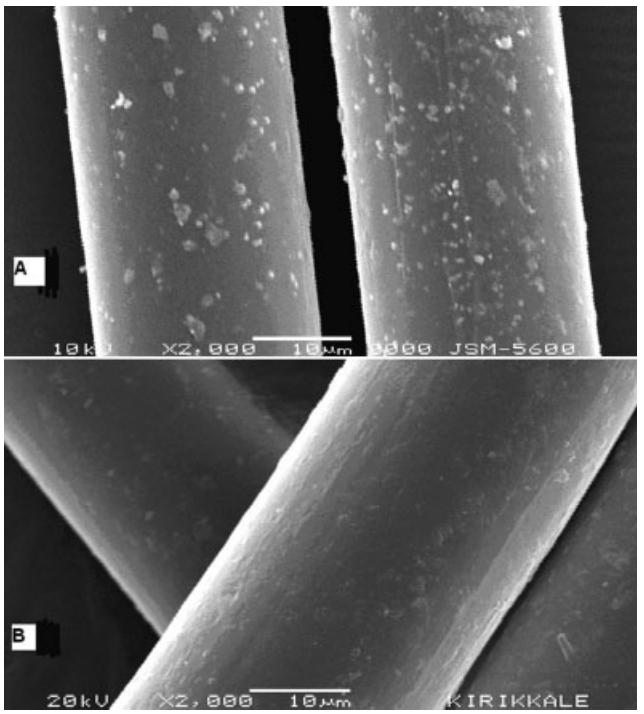

Figure 2 Scanning electron micrographs: (a) bare PET fibers; B: methacrylamide grafted (44%) PET fibers.

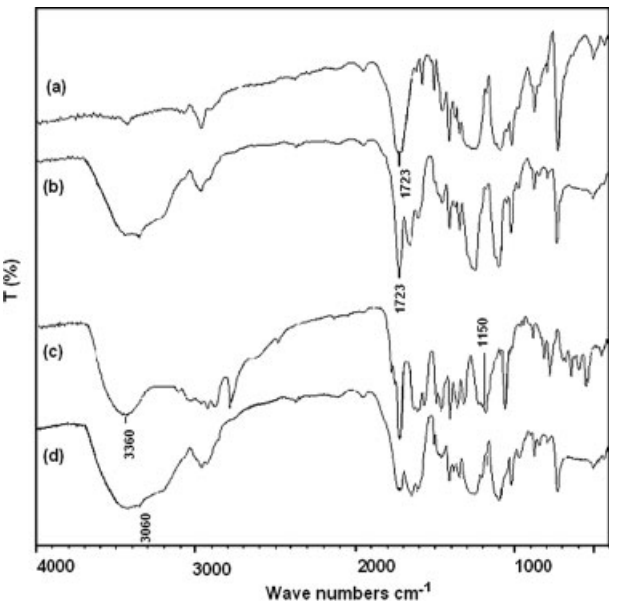

Figure 3 FTIR spectra: (a) PET fibers; (b) methacrylamide grafted (44.6%) PET fibers; (c) methacrylamide grafted (44.6%) and dye-ligand immobilized PET fibers; (d) lysozyme adsorbed PET-*g*-pMAAm-PB fibers.

The FTIR spectra of both bare PET and methacrylamide grafted PET fibers have the characteristic absorption bands of C=O and C=C at 1723 and 1400–1658 cm^{-1}, respectively. The characteristic stretching vibration band of aliphatic hydrogen bounded and aromatic hydrogen-bonded, C—H, observed at around 2967–2909 cm^{-1} and $\sim$ 3060 cm^{-1}, respectively, [Fig. 3(a)]. After grafting with MAAm, the spectra of the PET-*g*-pMAAm changed significantly [Fig. 3(a)]. The FTIR spectra of PET-*g*-pMAAm fiber have characteristic amide I and amide II adsorption bands at 3360 and 3441 cm^{-1}, respectively, due to the incorporation of methacrylamide grafting on the polymer structure, which was not observed in bare PET fibers. On the other hand $—SO_3$ stretching vibration at 1180 cm^{-1} band intensity of dye-ligand immobilized PET-*g*-pMAAm fibers is higher than that of PET-*g*-pMAAm fibers due to the incorporation of Procion Brown MX 5BR dye immobilized on the grafted polymer structure [Fig. 3(c)]. And the FTIR spectrum of lysozyme adsorbed PET-*g*-pMAAm-PB fiber is depicted in Figure 4(c).

The thermal stabilities of the PET, PET-*g*-pMAAm, and PET-*g*-pMAAm-PB were evaluated using thermogravimetric analysis. Figure 4 shows the TGA analysis of fibers. The bare PET fibers showed higher thermal stability compared to grafted counterpart fibers (i.e., PET-*g*-pMAAm) (Fig. 4). The initial weight loss of both bare and MAAm grafted PET fibers was due to the presence of small amount of moisture in the samples. It can be seen that TGA curve associated with the pMAAm grafted PET fibers shows greater slope than that of the bare PET

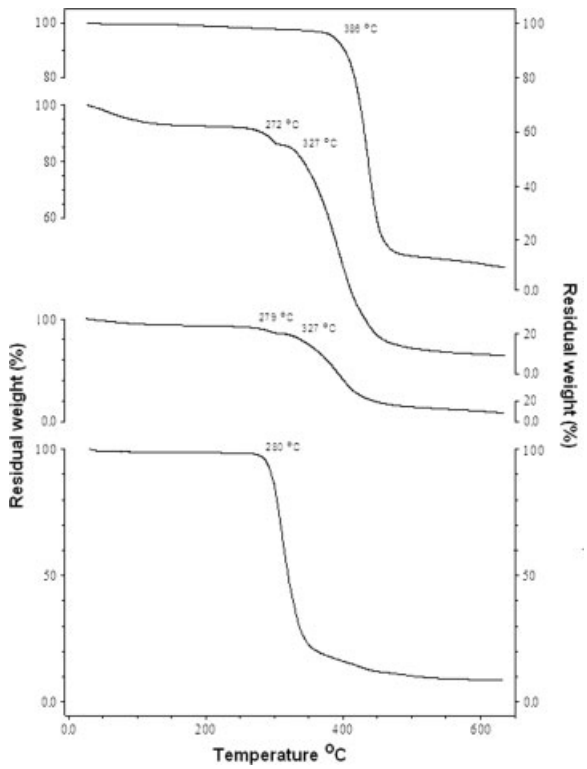

Figure 4 Thermogravimetric analysis of adsorbent: (a) PET; (b) PET methacrylamide grafted (44%) PET fibers; (c) methacrylamide grafted (44.6%) and dye-ligand immobilized PET fibers; (d) lysozyme adsorbed PET-*g*-pMAAm-PB fibers.

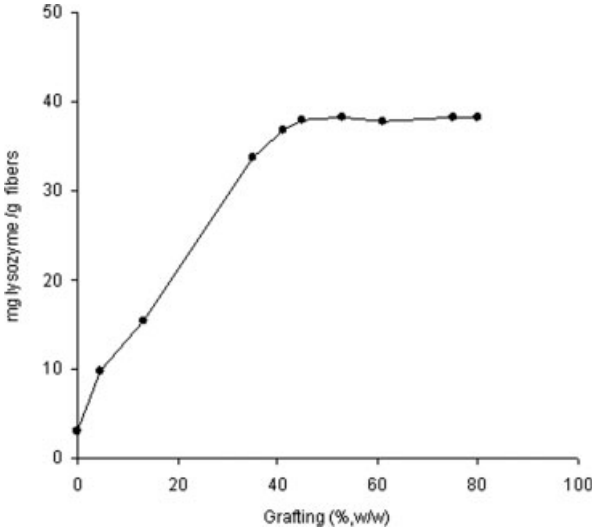

Figure 5 Effect of grafting percentage of pMAAM on the adsorption capacity of the PET-*g*-pMAAm-PB (44.6% MAAm) fibers; Initial concentration of lysozyme: 1.0 mg mL^{-1}; dye-ligand density: 417 μmol g^{-1}; Temperature: 25°C; pH: 7.5.

fibers, denoting a decrease in thermal stability of the grafted fibers. Thus, the grafting of pMAAm chains onto the PET fibers structure results a reduction of the thermal stability of the grafted-PET fibers compared to bare PET. It can be seen that after the complete degradation there were no polymer residue left, which was expected because of all compositions of the fiber are organic. As seen in this figure, the dye-ligand immobilized PET fibers showed higher thermal stability than of the PET-*g*-pMAAm fibers.

Effect of grafting percentage and dye-ligand density

Grafting improves the adsorption capacity and selectivity of fiber significantly by forming many reactive groups upon the polymer chains. The effect of grafted methacrylamide percentage on the lysozyme adsorption capacity of the PET-*g*-pMAAm-P**B** fibers is presented in Figure 5. As seen in this figure, an increase in the grafting percentage from 4.6% to 44.3% leads to increase up to 37.9 mg g^{-1} in lysozyme adsorption capacity of the PET-*g*-pMAAm-PR fibers. After this limit, the increase in grafting percentage does not significantly affect on the adsorption capacity of the PET-*g*-pMAAm-PR fibers. The rest of the study was carried out with 44.6% MAAm grafting percentage in PET-*g*-pMAAm-PB fibers. It should be noted that the amounts of immobilized dye were also increased as the grafting percentage increases but this increase is not proportional to graft percentage. The immobilized dye concentration was found to in the range of 378–694 μmol per g PET-*g*-pMAAm fibers. Procion Brown MX 5-BR is a dichlorotriazine dye (Fig. 1), which contains a sulfonate, a carboxyl a secondary amino and a hydroxyl groups. The binding affinity of the dye-ligand towards lysozyme may be due to an effect of a combination of mechanisms such as hydrophobic and/or ion-exchange interactions caused by the aromatic structures and acidic groups on the dye-ligand and by the side chain groups of amino acids of proteins. The dye-ligand has a semi hydrophobic nature that prefers to interact with hydrophobic as well as hydrophilic residues in proteins.

Effect of pH on lysozyme adsorption capacity

The protein binding affinity is controllable by the proton concentration in the electrostatic interaction-dominant process. As shown in Figure 6, the maximal adsorptive capacity on the PET-*g*-pMAAm-PB fibers occurred around in pH 7.5. Depending on its amino acid composition, a protein can have several charged groups at its pI value, the spatial arrangement of which is a function of primary, secondary, tertiary and quaternary structures. The isoelectric pH of lysozyme is 11.2. In general, maximum adsorption of protein is observed at its isoelectric pI value.[17] However, in the present study, the maximum adsorption was not at this pH, but had shifted toward less alkaline pH values. This could be due to preferential interactions between the lysozyme and the immobilized dye-ligand molecules on the polymeric fibers at pH 7.5. At this pH value, lysozyme is positively charged; ''only taking into account the surface charge distribution in amino acid side chains of lysozyme'' it has 17 positive charges and nine negative charges, resulting in a net eight positive

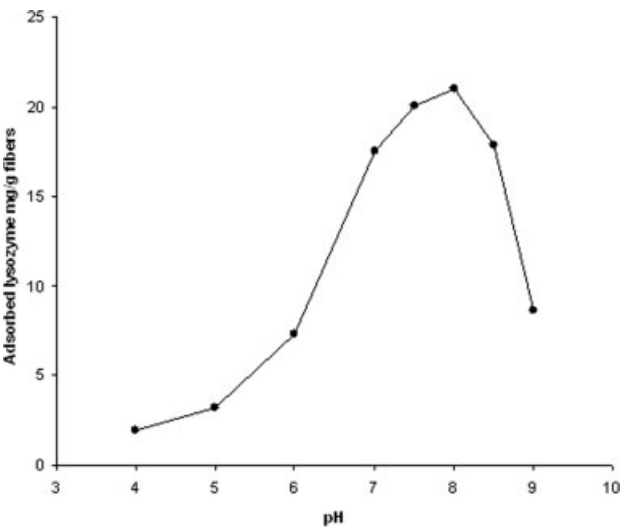

Figure 6 Effects of pH on lysozyme adsorption of the PET-*g*-pMAAm-PB (% 44.6 MAAm) fibers; Initial concentration of lysozyme: 0.5 mg mL^{-1}; Temperature: 25°C.

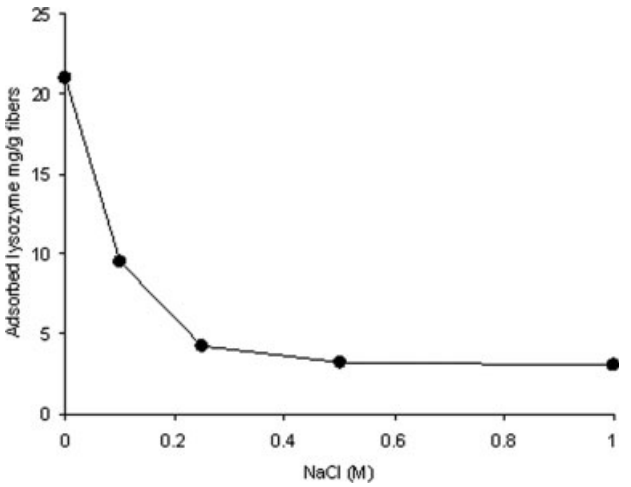

Figure 7 Effects of ionic strength on lysozyme adsorption on the PET-*g*-pMAAm-PB fiber; Initial concentration of lysozyme: 0.5 mg mL^{-1}; dye-ligand density: 417 μmol g^{-1}; Temperature: 25°C; pH: 7.5.

surface charge.[19,27] On the other hand, the dye ligand (i.e., Procion Brown MX 5BR) has two acidic groups (a sulfonate and a carboxyl) and each dye molecules bears two net negative charges. So positively charged lysozyme molecules could be easily interacted with negatively charged fibers, is electrostatically favorable.[28,29] It is similar to the results of lysozyme adsorption by immobilizing Cibacron Blue F3GA onto the thermosensitive polyacrylamide derivatives.[30] They reported that the lysozyme adsorption capacity was significantly influenced by the medium pH, and the adsorption capacity of lysozyme was 3.4 mg g^{-1} polymer at pH 7.0. So, in the following experiment, the adsorption of lysozyme on PET-*g*-pMAAm-PB fibers was conducted in pH 7.5.

Effect of ionic strength and temperature

The significance of ionic strength on adsorption of lysozyme on the PET-*g*-pMAAm-PB fibers was investigated by monitoring the adsorption process at different salt concentrations. The adsorption capacity of lysozyme remarkably decreased (from 20.98 to 4.22) mg g^{-1} when the concentration of NaCl was increased from 0.0 to 0.25 mol L^{-1}, which suggests that electrolyte greatly affects the interaction of lysozyme on PET-*g*-pMAAm-PB fibers (Fig. 7). As further increase of NaCl concentration, the adsorption capacity decreased slightly (from 3.25 to 3.04 mg g^{-1}) which might be due to the dominating of hydrophobic interaction[10,17,31] and/or the formation of more compact structures of the lysozyme molecules at high ionic strengths. In fact, hydrophobic and electrostatic interactions between lysozyme and PET-*g*-pMAAm-PB fibers may take place in the adsorption process, and cannot be neglected under certain circumstances.[17] In addition, pMAAm was grafted on the PET in brush form and then dye-ligand immobilized. Therefore, it could be suggested that the adsorption process could be taking place on the surface of the dye-ligand immobilized brush structure of the adsorbent. Thus, at high salt concentration, the brush structure may take a compact form due to the increased hydrophobic interaction of the dye-ligand molecules to each other. These phenomena could restrict the surface area of the adsorbent for interaction of lysozyme molecule with the ligand.

The lysozyme showed increased adsorption capacity with PET-*g*-pMAAm-PB fibers at higher temperatures and the results are presented in Table I. From 4 to 35°C, the adsorption capacity of the PET-*g*-pMAAm-PB fibers for lysozyme increased for about 81%. At higher temperature during the unfolding process, the proteins expose buried amino acid residues on the surface. Thus the contact area between the protein and the functional groups of the matrix should increase, resulting in an increase in the binding sites of the protein for the adsorbent at higher temperatures.

Effect of initial lysozyme concentration

Effect of initial lysozyme concentration, i.e., the amount of lysozyme adsorbed on the PET-*g*-pMAAm-PB fibers versus the initial concentration of lysozyme in the solution, is shown in Figure 8. As can be seen, lysozyme binding increases with its concentration in the adsorption medium until it approaches saturation at concentrations around 1.5 mg mL^{-1}. This is attributed to the binding sites

TABLE I
Langmuir Constants and Thermodynamic Parameters for Adsorption of Lysozyme on the PET-*g*-pMAAm-PB Fibers at Different Temperatures

	Langmuir constants				Thermodynamic parameters		
Temperature (K)	q_{exp} (mg g^{-1})	q_m (mg g^{-1})	$K_d \times 10^6$ (M)	R^2	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J $mol\ K^{-1}$)
277	28.54	33.03	0.17	0.996	−25.37		
288	33.67	37.88	0.11	0.998	−27.36		
298	43.90	49.11	7.85	0.997	−29.14		
308	51.65	52.39	94.30	0.998	−35.52	59.86	304.71

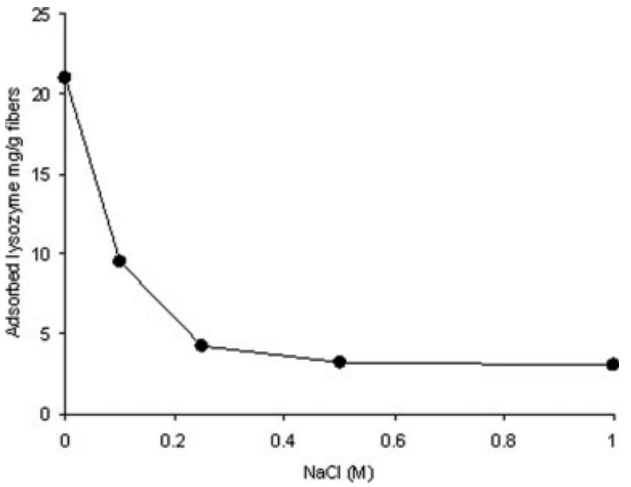

Figure 8 Effects of lysozyme initial concentration on lysozyme adsorption on the PET-*g*-pMAAm-PB fiber. pH: 7.5; Temperature: 25°C; dye-ligand density: 417 μmol g^{-1}.

of the PET-*g*-pMAAm-PB fibers become saturated. Maximum adsorption capacity was obtained as 43.9 mg g^{-1}. It should be noted that there was a small amount of lysozyme adsorption on the PET-*g*-pMAAm fibers (3.22 mg g^{-1}). The immobilization of the dye-ligand on the PET-*g*-pMAAm fibers significantly increased the lysozyme adsorption capacity about 13.6-fold of the PET-*g*-pMAAm-PB fibers. It is clear that this increase is due to ternary complex formation between PET-*g*-pMAAm fibers, dye-ligand and lysozyme molecules.[32]

Evaluation of adsorption isotherm parameters

The Langmuir model is based on the assumption of surface homogeneity such as equally available adsorption sites, monolayer surface coverage, and no interaction between adsorbed species and described by the following equation:

$$dq/dt = k_1 C(q_{\max} - q) - k_2 q \tag{6}$$

where C is the concentration of protein in solution, q is the amount of protein adsorbed on the fiber and $q_{\max}$ is the maximum adsorption capacity of the fibers. At equilibrium:

$$q = q_{\max} C/(K_d + C) \tag{7}$$

where K_d is the equilibrium constant.

Experimental equilibrium binding data were generated at different temperatures, the corresponding adsorption isotherms were constructed, and the Langmuir constants (q_{eq} and K_d) were determined from the semireciprocal plots fitted to the points by the least squares method. The correlation coefficients of semireciprocal plots (R^2) were greater than 0.996 for all the temperatures, indicating that the Langmuir model could be applied in this system. From the slopes, the maximum capacities (q_m) were found to be between 33.03 and 52.39 mg g^{-1} for lysozyme on the PET-*g*-pMAAm-PB fibers at different temperatures and these calculated q_m values were very close to the experimental q_{ex} values (Table I). Therefore, the adsorption of lysozyme onto PET-*g*-pMAAm-PB fibers could be described in terms of the Langmuir model. 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 adsorbent under specified experimental conditions. It should be noted that the equilibrium constant K_d, indicative to the strength of interactions between the adsorbed molecule and the adsorbing surface. At different temperatures, the K_d values were found to be between 0.17×10^{-6} and $94.30 \times 10^{-6} M$ for the adsorption of lysozyme on the PET-*g*-pMAAm-PB fibers and, a high binding affinity was obtained for the immobilized dye ligand.

To further understand the adsorption mechanism of lysozyme onto PET-*g*-pMAAm-PB fibers, thermodynamic analysis was carried out. The equilibrium association constant, ($K_a = 1/K_d$) extracted from the semireciprocal plot was then employed for the Van't Hoff plot analysis of log (K_a) versus the reciprocal of the temperature.

The value of ΔG and ΔS can be estimated from the relationships $\Delta G^0 = -RT \ln K_a$ and $\Delta G = \Delta H - T\Delta S$.

$$\ln K_a = (\Delta S/R) - (\Delta H/RT) \tag{8}$$

The results of the thermodynamic parameters for the adsorption of lysozyme by PET-*g*-pMAAm-PB fibers at different temperatures listed in Table I indicate that the enthalpy change of adsorption process is smaller than typical chelating bonding and ionic bonding, but higher than electrostatic and Van der Waals forces. The negative ΔG values for each temperature indicated that adsorption of lysozyme on the PET-*g*-pMAAm-PB fiber was a favorable process and those were ranged between −25.37 and −35.52 kJ mol^{-1}. From the thermodynamics aspect, the binding enthalpies and binding entropies in Table I verify that lysozyme adsorption procedures at pH 7.5 are endothermic and accompany the increasing of the system's entropy. The ΔS and ΔH values for the adsorption of lysozyme to PET-*g*-pMAAm-PB fiber were found 304.7 J mol^{-1} K^{-1} and 59.86 kJ mol^{-1}, respectively. The positive values of ΔS show the increased randomness at the solid/solution interface during adsorption. At lower temperatures, due to lower kinetic motion of the dye ligand, the value of the phase ratio will be different to that at higher temperatures. In addition, the $q_{\max}$

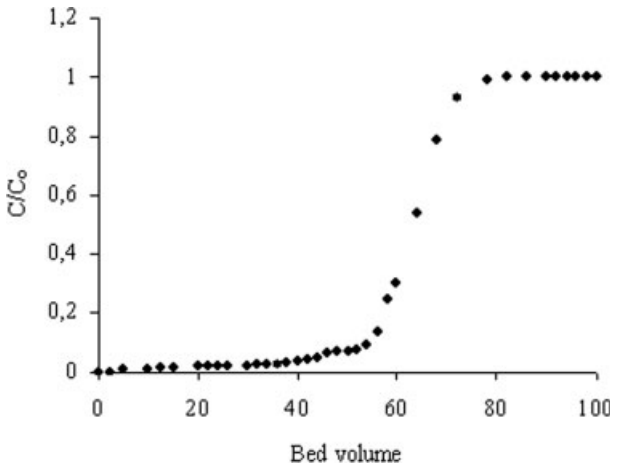

Figure 9 Breakthrough curves of lysozyme: Initial concentration of lysozyme: 1.5 mg mL^{-1}; dye-ligand density: 417 μmol g^{-1}; Temperature: 25°C; pH: 7.5.

and q_{exp} increased with the rise of temperature, implying the existence of chemical adsorption. Therefore, it can be concluded that multi-interactions are included in the adsorption process of lysozyme on PET-*g*-pMAAm-PB.

Breakthrough curves of lysozyme in column are shown in Figure 9. In general, a sharp breakthrough curve can be obtained if the adsorption isotherm is a favorable isotherm, such as Langmuir, as shown in Figure 9. The sharp breakthrough curve implies efficient adsorption performance and higher availability of column. The dynamic adsorption capacity of adsorbent in column was 33.8 mg g^{-1}, which was lower than batch adsorption capacity (43.9 mg g^{-1}). The adsorption time for determination of batch adsorption capacity is 3.0 h where the adsorption equilibration had been attained. However, the average retention time of lysozyme in column was only 2.0 h where the adsorption equilibration was not attained. Therefore, the continuous adsorption capacity is lower than batch adsorption capacity.

Evaluation of adsorption kinetic parameters

To investigate the mechanism of adsorption, the first-order and the second-order kinetics model were used to test dynamical experimental data. The first-order rate equation of Lagergren is one of the most widely used for the adsorption of solute from a liquid solution.[33] It may be represented as follows:

$$dq_t/dt = k_1(q_{eq} - q_t) \tag{9}$$

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

$$\log(q_{eq} - q_t) = \log q_{eq} - (k_1 t)/2.303 \tag{10}$$

A plot of log ($q_{eq}-q_t$) against t should give a straight line to confirm the applicability of the kinetic model. In a true first order process log q_{eq} should be equal to the intercept of a plot of log ($q_{eq}-q_t$) against t.

Ritchie proposed a second-order rate equation for the kinetic adsorption of gases on solids.[34] The second-order equation was applied for adsorption of solutes on the adsorbents.[17] The second-order equation based on adsorption equilibrium capacity may be expressed in the form:

$$1/q_t = 1/k_2 q_m t + 1/q_m \tag{11}$$

Table II lists the computed results obtained from the first-order and second-order kinetic models. The theoretical q_{eq} values for the PET-*g*-pMAAm-PB fibers were very close to the experimental q_{eq} values in the case of second-order kinetics. The correlation coefficients for the pseudosecond order kinetic model are higher than the pseudo first order kinetic model for all cases. These results suggest that the second-order mechanism is predominant and that chemisorption might be the rate-limiting step that controls the adsorption process. The rate-controlling mechanism may vary during the course of the adsorption process three possible mechanisms may be occurring.[29] There is an external surface mass transfer or film diffusion process that controls the early stages of the adsorption process. This may be followed by a reaction or constant rate stage and finally by a diffusion stage where the adsorption process slows down considerably.

TABLE II
Kinetics Parameters for the Adsorption of Lysozyme on to PET-*g*-pMAAm-PB Fibers

	First-order			Second-order		
q_{exp} (mg g^{-1})	q_{eq} (mg g^{-1})	$k_1 \times 10^2$ (min^{-1})	R^2	q_{eq} (mg g^{-1})	$k_2 \times 10^2$ (g mg^{-1} min^{-1})	R^2
43.90	75.22	5.64	0.950	41.73	5.29	0.999

Desorption of lysoyme and reusability of PET-*g*-pMAAm fibers

Appropriate to economic restraints, there is a growing interest in the preparation and use of effective low-cost and reusable adsorbents.[35] Desorption of the adsorbed lysozyme from the PET-*g*-pMAAm fibers was studied in a batch system. Elution of lysozyme was studied with using phosphate buffer containing 1.0*M* NaCl. Lysozyme adsorbed PET-*g*-pMAAm fibers were placed within the elution medium and stirred continuously (at stringing rate 150 rpm) for 2.0 h at room temperature. To show the reusability of the PET-*g*-pMAAm-PB fibers, adsorption-desorption cycle of lysozyme was repeated five times by using the same PET-*g*-pMAAm-PB fibers. At the end of five adsorption-elution cycle, there was no remarkable reduce in the adsorption capacity. PET-*g*-pMAAm-PB fibers are stable when subjected to sanitization with sodium hydroxide after repeated adsorption-elution cycles.

Fluorescence spectrometer was used to assess the possible structural changes of lysozyme during the adsorption and desorption process. The fluorescence spectra of lysozyme sample obtained from the elution step was recorded. When the excitation wavelength is 280 nm both tryptophan and tyrosine amino acid residues, in protein has fluorescence emission. While at the excitation wavelength of 295 nm, only tryptophan amino acid residues has fluorescence emission. 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 fluoresecence spectra of the sample withdrawn from the elution step was 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-ligand affinity chromatography can be applied to lysozyme separation without any conformational changes or denaturation.

Adsorption and Purification of lysozyme from egg white

Chicken egg white is a mixture of proteins. Among the proteins in egg whites, ovalbumin, conalbumin, and ovomucoid are the major components constituting 54, 12–13, and 11%, respectively. The content of lysozyme as a minor component in chicken egg white is about 3.4%. The purity of the lysozyme eluted from PET-*g*-pMAAm-PB fibers was determined by HPLC. The purity of the eluted lysozyme, as determined by HPLC, was 84% with a recovery of 73%. At pH 7.5, lysozyme has positive charge and other egg white proteins are negatively charged. Therefore, only lysozyme could be captured by the chromatographic matrix (PET-*g*-pMAAm-PB fibers) under the given experimental conditions. The dye-ligand immobilized PET-*g*-pMAAm fibers provided an efficient single step method for purification of lysozyme from diluted egg white. The specific activity of the purified lysozyme with PET-*g*-pMAAm fibers was 44.835 U mg^{-1}.

CONCLUSIONS

In this study, PET fibers were grafted with methacrylamide using benzylperoxide as initiator. The amide groups of the grafted methacrylamide were utilized for the ligand immobilization sites. SEM measurements confirmed the growth of methacrylamide brushes on the surface of the PET fibers, and reflectance FTIR spectroscopy verified the efficacy of the grafting procedures. After immobilization of the dye-ligand (i.e., Procion Brown MX-5BR) on the methacrylamide brushes, the derivatized fibers were used for adsorption and purification of lysozyme. It has been shown that the grafting percentage, dye-ligand density, pH, ionic strength, and temperature can have important effects on the adsorption equilibrium. The pH of the medium has an important effect, and there is a preferential interaction between lysozyme and PET-*g*-pMAAm-PB fiber at pH 7.5. The Langmuir adsorption isotherm model best described the data. The theoretical q_{eq} values for all the tested adsorbent systems were very close to the experimental q_{eq} values in the case of second-order kinetics. To examine the effect of adsorption/desorption conditions on conformational changes of lysozyme molecules, fluorescence spectrophotometry was employed. It appears that the dye-ligand chromatography can be applied for the adsorption of lysozyme without causing any denaturation. Finally, Procion Brown MX 5-BR immobilized PET-*g*-MMAm fibers promoted an efficient method to purify lysozyme, assuring a purity of about 84% with recovery about 73%.

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