ELSEVIER

Journal of Chromatography A, 799 (1998) 83–91

JOURNAL OF CHROMATOGRAPHY A

Dye–ligand and metal chelate poly(2-hydroxyethylmethacrylate) membranes for affinity separation of proteins

M. Yakup Arıca[a], H. Nur Testereci[b], Adil Denizli[c,*]

[a]*Department of Biology, Kirikkale University, Kirikkale, Turkey*
[b]*Department of Chemistry, Kirikkale University, Kirikkale, Turkey*
[c]*Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey*

Received 14 July 1997; received in revised form 13 October 1997; accepted 21 October 1997

Abstract

Cibacron Blue F3GA was covalently immobilized onto poly(2-hydroxyethyl methacrylate) (pHEMA) membranes via the nucleophilic reaction between the chloride of its triazine ring and the hydroxyl group of pHEMA. Then, Fe^{3+} ions were complexed by chelation with the immobilized Cibacron Blue F3GA molecules. Different amounts of Fe^{3+} ions were loaded on the membranes by changing the concentration of Fe^{3+} ions and pH of the reaction medium. Membranes with or without Fe^{3+} were used in the adsorption of glucose oxidase, catalase and bovine serum albumin. The adsorption capacities of these membranes were determined by changing pH and the concentration of the proteins in the adsorption medium. The adsorption phenomena appeared to follow a typical Langmuir isotherm. The maximum capacities (q_m) of the Fe^{3+} complexed membranes for glucose oxidase, catalase and bovine serum albumin ($8.70\cdot10^{-3}$ μmol m^{-2}, $2.15\cdot10^{-3}$ μmol m^{-2} and $2.21\cdot10^{-3}$ μmol m^{-2}) were greater than those of the untreated membranes ($6.79\cdot10^{-3}$ μmol m^{-2}, $1.34\cdot10^{-3}$ μmol m^{-2} and $1.94\cdot10^{-3}$ μmol m^{-2}) respectively. The nonspecific adsorption of the enzymes and the protein on the pHEMA membranes was negligible.

Keywords: Affinity membranes; Membranes; Metal chelate affinity chromatography; Poly(2-hydroxyethyl methacrylate) membranes; Affinity adsorbents; Proteins; Enzymes; Metal chelates; Iron; Cibacron Blue F3GA

1. Introduction

The developments in biotechnology and genetic engineering have accelerated the efforts in large-scale, industrial production of various proteins and enzymes. High rate collection of proteins and enzymes from biological fluids such as liquid culture medium and serum requires simple chromatographic separation techniques [1–3].

Triazine dyes are perhaps the most promising pseudo-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, stability and adsorbent capacity [4,5]. The only drawback of textile dyes appears to be their moderate selectivity for target enzyme, this problem will be overcome by introducing new selectively interacting materials on the basis of their affinities for chelated transition metal ions. The separation is based on differential binding abilities of the proteins or enzymes to interact with chelated metal ions to a solid carrier [6,7]. Metal chelate affinity chromatography of proteins, with metal

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01079-0

chelate linked to Sepharose, was first described by Porath et al. [8]. They reported a model system using Zn^{2+} and Cu^{2+} columns in tandem for the fractionation of human serum proteins. Plasminogen activators from both normal tissue (human uterus) and human melanoma cells have been isolated by metal chelate affinity chromatography [9,10] as have nucleoside diphosphatase [11], human lactoferrin [12], lectin [13], interferon [14], carboxypeptidase B [15] and bovine serum albumin [7].

In addition to the development of the novel adsorbent for affinity columns, improvements in adsorbent geometry have been undertaken to process a large quantity of proteins rapidly. The use of affinity membranes in the chromatographic separation has been proposed as an effective alternative for diffusion-limited separation using a conventional gel bead [6,16–18]. In recent years, microporous membranes, generally used for separation of whole cells, proteins and microbial enzymes and several applications have already been reported [19–21].

Poly(2-hydroxyethyl methacrylate) is a hydrogel, since the matrix retains a large amount of water and it possesses a high mechanical strength. These properties are also important in its use as support material in affinity chromatography and bioreactors [22,23]. The presence of hydroxyl groups offers attachment sites for enzymes and proteins via activation and derivatization by introduction of a variety of ligands [24]. It has been used in previous enzyme immobilization studies either by entrapment into or by covalent binding onto its membrane [22,25].

The purpose of this study was to prepare an affinity membrane sorbent containing Cibacron Blue F3GA (CB) and Fe^{3+} ions (in chelate form) for dye affinity and metal chelate affinity separation of proteins. Three different proteins were selected as model adsorbates; glucose oxidase (GOD) as a flavoenzyme which contains two tightly bound flavine adenine dinucleotide (FAD) cofactors (phosphate groups present on the FAD group shows affinity towards iron ions), catalase (CAT) as a heme containing metallo-enzyme (has also a protoporphyrin group and it shows specific interaction with iron ions) and bovine serum albumin (BSA) as a simple protein and the effect of their structural difference on the adsorption properties of the affinity membrane supports is described.

2. Experimental

2.1. Materials

Glucose oxidase (GOD, oxygen 1-oxido reductase, E.C.1.1.3.4, Type II from *Aspergillus niger*), catalase (CAT, hydrogen peroxide oxido reductase, EC.1.11.1.6) from bovine liver bovine serum albumin (BSA, Fraction V) and Cibacron Blue F3GA were purchased from Sigma (St. Louis, MO, USA) and used as received. 2-Hydroxyethyl methacrylate (HEMA) was obtained from Sigma and distilled under reduced pressure in the presence of hydroquinone and stored at 4°C until use. α′-α′-Azobisisobutyronitrile (AIBN) was purchased from Fluka (Buchs, Switzerland) and used as received. All other chemicals were of reagent grade and were purchased from Merck (Darmstadt, Germany).

2.2. Membrane preparation

The poly(2-hydroxyethyl methacrylate), pHEMA, membrane was prepared as previously described [26]. The membrane preparation mixture (5.0 ml) contained 2.0 ml (HEMA), 5 mg AIBN as polymerization initiator and 3.0 ml 0.1 *M* $SnCl_4$. The mixture was then poured into a round glass mould (diameter 4.5 cm) and exposed to ultraviolet radiation for 10 min, while a nitrogen atmosphere was maintained in the mould. The membrane was washed several times with distilled water and cut into circular pieces (diameter 0.5 cm) with a perforator.

2.3. Dye attachment to pHEMA membrane

The hydroxyl groups of pHEMA membrane were derivatized by reaction with Cibacron Blue F3GA (CB). CB (300 mg) was dissolved in 10 ml water. This triazine dye solution was transferred to pHEMA membrane pieces (diameter 0.5 cm, thickness ~0.06 cm) in 90 ml distilled water, and then 4 g of NaOH were added. The medium was heated at 80°C in a sealed reactor and was stirred magnetically for 4 h. The membrane pieces were washed several times with distilled water and methanol until all the physically attached dye was removed. They were then stored at 4°C until use.

2.4. Incorporation of Fe^{3+} ions to dye attached pHEMA membranes

The effect of pH on the chelate formation of the CB derived pHEMA membranes with Fe^{3+} ions was investigated in a batch system at 25°C. A 10 ppm solution of Fe^{3+} ions was prepared in universal buffer solution in the pH range 2.0–5.5. The CB attached pHEMA membranes were transferred to buffer solution (20 ml) and magnetically stirred for 1 h.

The effect of initial Fe^{3+} ion concentration on chelate formation was studied at pH 5.0 as described above except that universal buffer solution containing 10–300 ppm Fe^{3+} ions was used. The concentration of the Fe^{3+} ions in the resulting solution was determined with atomic absorption spectrophotometry (AAS) (GBC 932 AA, Australia).

The Fe^{3+} leakage from CB-attached and Fe^{3+} derived pHEMA membranes was determined in a solution at a pH value in the range of 4–8 and containing 1.0 *M* NaSCN (pH 8.0). The solution containing the derivatized pHEMA membrane disks was stirred for 24 h at room temperature. After this period, the leached Fe^{3+} was determined in these solutions by using AAS.

2.5. Adsorption studies with GOD, CAT and BSA

GOD, CAT and BSA adsorption of the dye derivatized (pHEMA-CB) and metal chelated (pHEMA–CB–Fe^{3+}) membrane disks was studied at various pH values, in either 0.1 *M* acetate (5.0 ml, pH 4.0–5.5) or in 0.1 *M* phosphate buffer (5.0 ml, pH 6.0–8.0). The initial GOD, CAT and BSA concentrations were 3.0 mg ml^{-1} in the corresponding buffer. The adsorption experiments were conducted for 2 h at 25°C while continuously stirring. At the end of this period, membranes were removed from each enzyme and protein solution and were washed with same buffer three times. They were then stored at 4°C in fresh buffer until use.

In order to determine the adsorption capacities of derived pHEMA membranes, the concentrations of GOD, CAT and BSA in the solutions were varied between 0.5 and 5.0 mg ml^{-1}. The adsorption experiments were carried out for GOD at pH 7.0, for CAT at pH 6.8 and for BSA at pH 5.0. It should be noted that all protein adsorption curves are averages of at least duplicated experiments.

The amount of adsorbed proteins was obtained by using the following equation.

$$Q = [C_0 - C)V]/A \quad (1)$$

where, Q is the amount of proteins adsorbed onto unit surface area of the pHEMA membrane (mg m^{-2}); C_0 and C are the concentrations of the protein solutions in the initial solution and in the aqueous phase after adsorption, respectively (mg ml^{-1}), V is the volume of the aqueous solution (ml), and A is the surface area of the pHEMA membrane (m^2).

2.6. Desorption of enzymes and protein from derived pHEMA membrane

In order to determine the reusability of the CB attached and iron derived pHEMA membrane, GOD, CAT and BSA adsorption and desorption cycle were repeated five times using the same pHEMA matrix. The enzyme and protein desorption from pHEMA–CB and pHEMA–CB–Fe^{3+} membranes was carried out with 1.0 *M* NaSCN (pH 8.0) and stirred magnetically for 2 h at room temperature. The membranes were removed and washed several times with 0.1 *M* phosphate buffer (pH 7.0), and then reused in enzymes and protein adsorption.

The desorption ratios of the proteins and Fe^{3+} were then calculated by using the following expression:

$$\text{Desorption ratio} = \frac{[\text{amount of protein (or } Fe^{3+}\text{) released} \times 100]}{[\text{amount of protein (or } Fe^{3+}\text{) adsorbed on the membrane}]} \quad (2)$$

2.7. Determination of adsorption efficiency

The amount of protein in the crystalline enzymes and protein preparation and in the wash solution was determined by spectrofluorimetry (excitation at 280 nm and emission at 340 nm) using a Shimadzu (Model RF 5000) spectrofluorimeter. A calibration curve constructed with BSA solution (0.02–0.2 mg ml^{-1}) was used in the calculation of enzyme concentration.

Protein concentration (mg ml^{-1})
= fluorescence intensity at 340 nm/slope (3)

2.8. Elemental analysis

The amount of Cibacron Blue F3GA attached covalently to pHEMA membrane was evaluated from the elemental analysis device (Leco, CHNS-932, USA).

3. Results and discussion

3.1. Cibacron Blue F3GA derived pHEMA membrane

Dye immobilization requires an inert, hydrophilic support which possesses chemically modifiable groups. Most frequently used matrices are the naturally occurring polysaccharide polymers: agarose, dextran and cellulose. However, these natural polymers can undergo biological degradation and, for some applications, they show insufficient mechanical strength and porosity [21,27]. In comparison with other supports, the synthetic hydrophilic polymer pHEMA, due to its synthetic nature, is very inert toward microbial degradation and resistant to many chemicals. Its porosity can be modified by varying the concentration of ions and pore forming agent in the polymerization medium. Preparation and characterization details of the pHEMA membrane were given in our previous papers [23,26].

Cibacron Blue F3GA is a monochlorotriazine dye (Fig. 1), which contains three acidic sulfonate groups and four basic primary and secondary amino groups; the binding of Fe^{3+} ions to the Cibacron Blue F3GA molecules occurs especially through oxygen and nitrogen atoms. The strong binding of Cibacron Blue F3GA to proteins occurs largely at binding sites for substrates, coenzymes and other prosthetic groups [28,29].

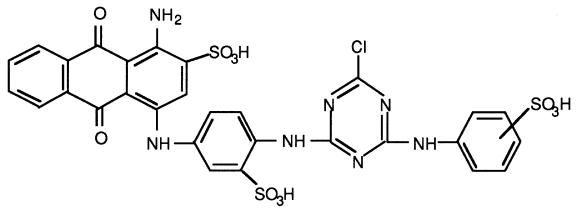

Fig. 1. Chemical structure of Cibacron Blue F3GA.

Adsorption equilibria were achieved gradually in about 1 h. The SEM pictures of the pHEMA membrane previously reported [23] show that the membrane has a highly open pore structure which may lead to a large internal surface area (implying high adsorption capacity) with low diffusional resistance in the matrix (implying high adsorption rates). Achieving both high adsorption capacities and rates were the main concerns in the preparation of affinity membranes for enzyme and protein adsorption.

pHEMA and pHEMA–CB membranes were subjected to elemental analysis. The amount of CB attached to the membrane was calculated from this data, (by considering the stoichiometry), to be 10.7 mmol CB m^{-2}.

Studies aimed at detecting leakage of CB and Fe^{3+} from the CB attached and Fe^{3+} derived pHEMA membrane 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 CB molecules and Fe^{3+} ions from pHEMA membranes.

3.2. Cibacron Blue F3GA–Fe^{3+} attached pHEMA membrane

As seen in Fig. 2, the adsorption of Fe^{3+} ions on the pHEMA–CB membranes increased with increasing pH, but this relation levelled off at around pH 5.0. The nonspecific adsorption of Fe^{3+} ions was about 6 mg m^{-2} for the plain of the pHEMA membrane, while the specific adsorption of Fe^{3+} ions was much higher (135 mg m^{-2} membrane) than nonspecific adsorption.

Fig. 3 shows the effects of Fe^{3+} ion concentration on the amount of Fe^{3+} ions adsorbed (chelated) on both the pHEMA and pHEMA–CB membranes. The amount of adsorbed Fe^{3+} ions on the pHEMA–CB membranes increased linearly up to 100 ppm Fe^{3+} ion concentration, beyond which a plateau was observed. A value of 473 mg Fe^{3+} m^{-2} membrane was reached. This was 12 mg Fe^{3+} m^{-2} for the plain pHEMA membranes.

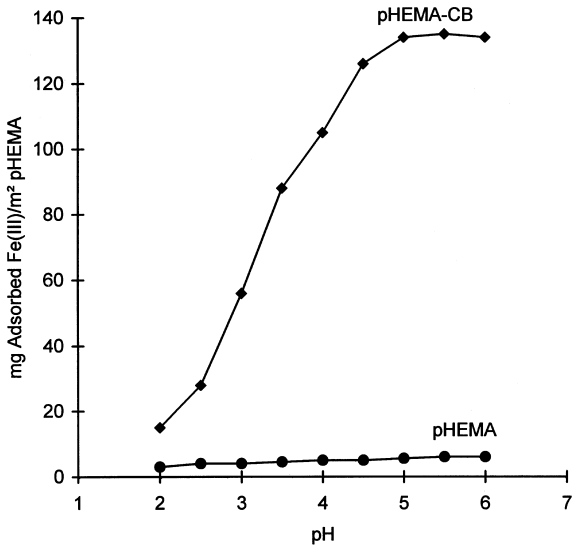

Fig. 2. Incorporation of Fe^{3+} ions onto pHEMA and pHEMA–CB membranes as a function of pH; initial Fe^{3+} concentration 10 ppm.

3.3. Adsorption efficiency of enzymes and protein

Three different proteins were selected as model adsorbates; GOD (M_r 144 000) as a flavoenzyme containing two FAD molecules (phosphate groups present on the FAD group shows affinity towards iron ions), CAT (M_r 240 000) as a metallo-enzyme (also has a protoporphyrin group and it shows specific affinity to iron ions) and BSA (M_r 60 000) as a simple protein. In order to ascertain the effect of their size and structural difference on the adsorption process similar experiments were carried out by using pHEMA–CB and pHEMA–CB–Fe^{3+} membranes.

The optimal pH values for adsorption of GOD, CAT and BSA onto pHEMA–CB and pHEMA–CB–Fe^{3+} membranes were investigated in the pH range 4.0–8.0. As observed in Fig. 4, with pHEMA–CB–Fe^{3+} membrane 872 mg m^{-2} GOD adsorption was obtained while with pHEMA–CB this was 662 mg m^{-2}. The adsorbed GOD value of each type of membrane was quite close for all the tested pH range. It is well known that Cibacron Blue F3GA is a group affinity dye and also possesses a high affinity to FAD [4,30,31]. It has been used in the purification of several flavoproteins exhibiting tightly bound FAD [32–34]. GOD was found to be a dimeric flavoprotein and one molecule of FAD is tightly bound per enzyme dimer [35]. The high adsorption of GOD on pHEMA–CB and pHEMA–CB–Fe^{+3} in the tested pH range may be due to the high affinity binding sites of GOD brought by FAD.

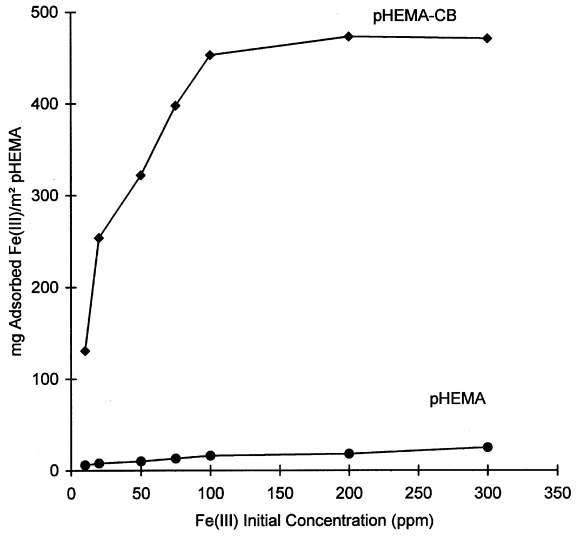

Fig. 3. Incorporation of Fe^{3+} ions onto pHEMA and pHEMA–CB membranes as a function of Fe^{3+} ion concentration at pH 5.0.

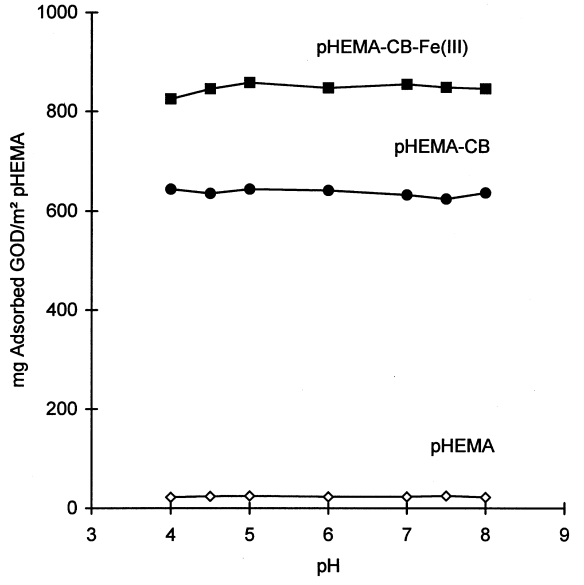

Fig. 4. Effect of pH on GOD adsorption; GOD concentration: 3.0 mg ml; CB loading: 10.7 mmol m^{-2}; Fe^{+3} loading: 473 mg m^{-2}.

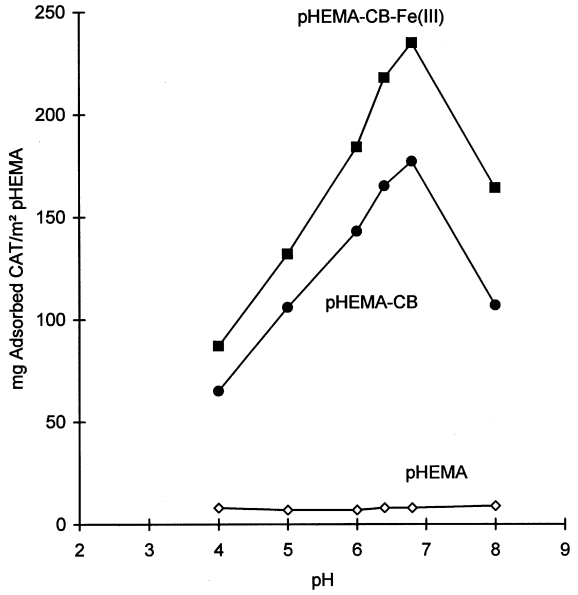

Fig. 5. Effect of pH on CAT adsorption; CAT concentration: 3.0 mg ml; CB loading: 10.7 mmol m^{-2}; Fe^{+3} loading: 473 mg m^{-2}.

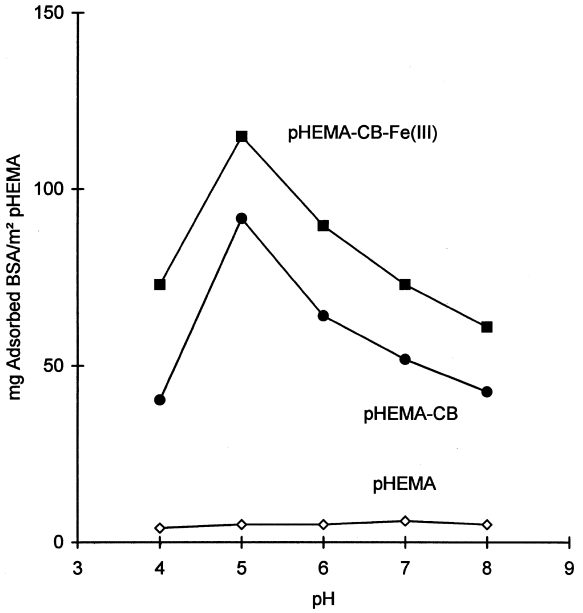

Fig. 6. Effect of pH on BSA adsorption; BSA concentration: 3.0 mg ml; CB loading: 10.7 mmol m^{-2}; Fe^{+3} loading: 473 mg m^{-2}.

In the case of CAT (Fig. 5), the maximum adsorptions onto pHEMA–CB–Fe^{3+} and pHEMA–CB membranes were 235 mg m^{-2} and 161 mg m^{2} at 6.8, respectively. Significantly lower adsorption was obtained for both pHEMA preparation in alkaline and acidic pH regions.

The maximum BSA adsorptions for pHEMA–CB–Fe^{3+} and pHEMA–CB membranes were 115 mg m^{-2} and 92 mg m^{-2}, respectively, and were obtained at pH 5.0 (Fig. 6).

It has been shown that proteins have no net charge at their isoelectric points, and therefore the maximum adsorption from aqueous solutions is usually observed at their isoelectric point [36]. The isoelectric (p*I*) values of GOD, CAT and BSA are 4.0, 6.4 and 4.9, respectively.

In the present study, there was no optimum pH for the GOD adsorption on pHEMA–CB and pHEMA–CB–Fe^{3+} membranes. The maximum adsorption of CAT was found close to its p*I* 6.4, it was slightly shifted (0.4 pH unit) to rather neutral pH value. In the case of BSA, the maximum adsorption was observed at its p*I* value. These specific interactions may result from the cooperative effect of different mechanisms such as hydrophobic interactions and/or ion-exchange effects, caused by the aromatic structure and sulfonic acid on the CB and the amino acid side-chains groups of the proteins [36].

An adsorption isotherm is used to characterize the interaction of each enzyme and protein with the adsorbents. This provides a relationship between the concentration of protein in the solution and the amount of protein adsorbed on the solid-phase when the two phases are at equilibrium. The Langmuir adsorption model assumes that the molecules are adsorbed at a fixed number of well-defined sites, each of which can only hold one molecule. These sites are also assumed to be energetically equivalent, and distant to each other so that there are no interactions between molecules adsorbed to adjacent sites [37].

Semi-reciprocal plots of the experimental data for the adsorption of GOD, CAT and BSA are presented for both pHEMA–CB and pHEMA–CB–Fe^{3+} membranes in Figs. 7 and 8, respectively. The corresponding semi-reciprocal transformations of the equilibrium data for enzymes and protein and dye derived and Fe^{3+} attached pHEMA membranes gave

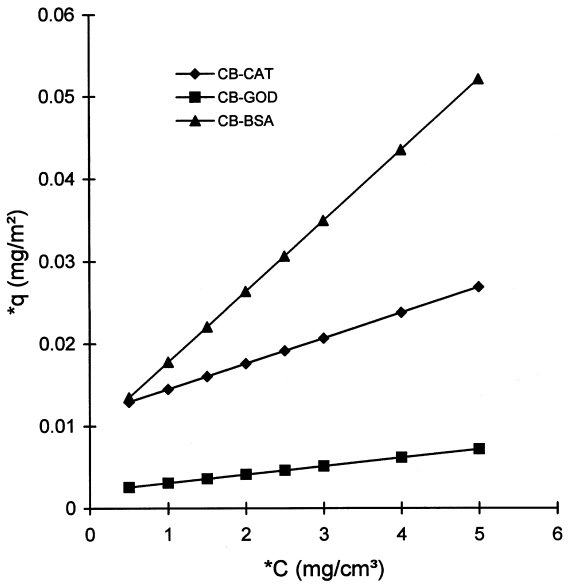

Fig. 7. Adsorption isotherm of GOD, CAT and BSA with pHEMA–CB membrane: initial proteins concentration between: 0.5–5.0 mg ml; CB loading: 10.7 mmol m^{-2}.

rise to a linear plot, indicating that the Langmuir model could be applied in these systems and which were described by the equation:

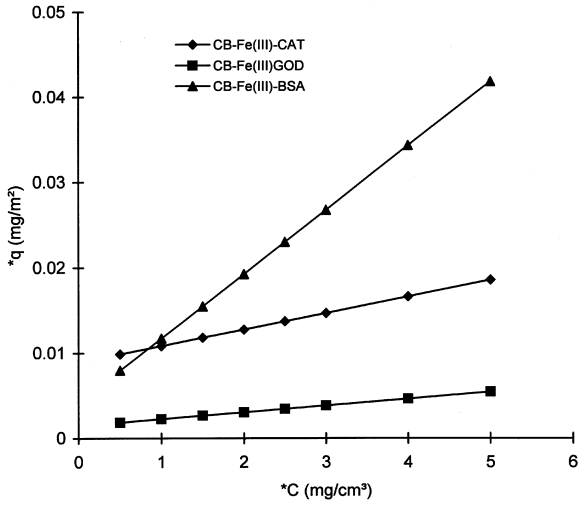

Fig. 8. Adsorption isotherm of GOD, CAT and BSA with pHEMA–CB–Fe^{3+} membrane: initial proteins concentration between: 0.5–5.0 mg ml; CB loading: 10.7 mmol m^{-2}; Fe^{+3} loading: 8.48 μmol m^{-2} membrane.

$$dq/dt = k_1C(q_m - q) - k_2q \quad (5)$$

where C is the concentration of adsorbate in solution, q is the solid-phase concentration of the adsorbed molecules and q_m is the maximum capacity of the adsorbent. Eq. (5) leads to

$$q^* = Q_mC^*/(k_d + C) \quad (6)$$

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

The semi-reciprocal plot of C^*/q^* versus C^* was employed to generate the intercept of k_d/q_m and the slope of $1/q_m$. The maximum capacity (q_m) and the dissociation constant (k_d) data for the adsorption of GOD, CAT and BSA to the pHEMA–CB and pHEMA–CB–Fe^3 membranes were obtained from the experimental data. The maximum capacities (q_m) were $6.79\cdot10^{-3}$ μmol m^{-2} and $8.70\cdot10^{-3}$ μmol m^{-2} for GOD, $1.34\cdot10^{-3}$ μmol m^{-2} and $2.15\cdot10^{-3}$ μmol m^{-2} for CAT, and $1.94\cdot10^{-3}$ μmol m^{-2} and $2.21\cdot10^{-3}$ μmol m^{-2} for BSA when pHEMA–CB and pHEMA–CB–Fe^{3+} membranes respectively were used in the adsorption tests. The order of q_m value for both case is as follows: GOD > CAT > BSA. The amount of Cibacron Blue F3GA and Fe^{3+} ions loading on the surface of CB derivatized pHEMA membrane were 10.7 mmol m^{-2} and 8.48 μmol m^{-2} (473 mg m^{-2}), respectively. The binding ratios of the dye and proteins were all found to be of the same order (10^6 dye molecules per protein molecule) but GOD had a higher binding ratio than CAT or BSA. For the pHEMA–CB–Fe^{3+} membrane the binding ratios were calculated for Fe^{3+} ions and found to be $9.75\cdot10^2$, $4.04\cdot10^3$, and $3.94\cdot10^3$ Fe^{3+} ions molecules for GOD, CAT and BSA, respectively. Incorporation of Fe^{3+} ions to the pHEMA–CB membrane leads to a significant increase in the maximum capacity of the membranes to enzymes and protein. The q_m values of GOD, CAT and BSA were increased about 28%, 60% and 13%, respectively.

It is clear that this increase is due to ternary complex formation between triazine dye, Fe^{3+} ions and proteins molecules (i.e. Fe^{3+} ions promote the adsorption of GOD, CAT and BSA). The binding of proteins to transition metals occurs via the electron-donating side chains of residues such as histidine and cysteine, which substitute water molecules coordi-

nated to the metal. The number of binding sites on the protein (histidine and cysteine residues), the chelate–polymer structure and pH influence metal affinity protein precipitation [6,7]. Catalase is an iron containing metalloenzyme and this may cause a specific interaction with the iron ions on the membrane through the iron binding sites of the catalase. This high specific interaction may lead to an increase in the q_m value (about 60%) of the Fe^{3+} attached pHEMA–CB membrane.

The k_d values of the three proteins were derived from the semi-reciprocal plots and were found to be $1.39 \cdot 10^{-5}$ *M* and $0.75 \cdot 10^{-5}$ *M* for GOD, $1.52 \cdot 10^{-5}$ *M* and $1.92 \cdot 10^{-5}$ *M* for CAT and $1.78 \cdot 10^{-5}$ *M* and $0.93 \cdot 10^{-5}$ *M* for BSA, with pHEMA–CB and pHEMA–CB–Fe^{3+} membranes systems, respectively. After Fe^{3+} incorporation, an increase in the maximum adsorption capacity of the pHEMA–CB membrane for GOD, CAT and BSA was obtained, while a slight increase in the dissociation constants of CAT was observed. The reduction in the dissociation constants of GOD and BSA was about 39% and 98.7%, respectively. This might be due to the lower molecular mass of proteins (GOD, 144 000; BSA, 67 000), leading to better affinity than for the higher-molecular-mass protein in this case; CAT has a molecular mass of 240 000.

Note that a wide variety of sorbents with a wide range of absorbtion capacities were reported in the literature for albumin adsorption. Denizli et al. found 41 mg m^{-2} adsorption capacity with Congo Red attached monosize pHEMA–MMA microspheres [7]. Tuncel et al. reached an adsorption capacity of 40 mg BSA m^{-2} with the polylvinyl alcohol coated CB immobilized monosize polystyrene microspheres [38]. Zeng and Ruskenstein have reported 139 mg HSA m^{-2} with the CB attached microporous chitosan membranes [39]. The maximum adsorptions for GOD 872 mg m^{-2}, for CAT 235 mg m^{-2} and for BSA 115 mg m^{-2} were achieved with pHEMA–CB–Fe^{3+} sorbent system developed in this study which was quite comparable with the related literature.

3.4. Desorption of enzymes and protein from affinity membranes

Desorption of adsorbed GOD, CAT and BSA from dye-derived pHEMA membrane was carried out in a batch system. pHEMA–CB or pHEMA–CB–Fe^{3+} and enzymes or protein preparation were placed within the desorption medium containing 1.0 *M* NaSCN (pH 8.0) at room temperature for 2 h as described above and was repeatedly used in adsorption of GOD, CAT and BSA. The enzyme and protein adsorption capacities did not change during five successive adsorption–desorption cycles of the pHEMA–CB and pHEMA–CB–Fe^{3+} preparation. Adsorption capacities of the preparations did not significantly change during these adsorption–desorption cycles. These results showed that CB and CB–Fe^{3+} derived novel affinity pHEMA membranes can be repeatedly used in enzyme and protein adsorption studies without detectable losses in their initial adsorption capacities.

4. Conclusion

A microporous pHEMA membrane containing CB and Fe^{3+} ions was prepared by UV-initiated photopolymerization of HEMA. The results showed that different types of protein were adsorbed with high affinity interaction on the membranes. The pHEMA membrane modified by attachment of CB and Fe^{3+} ions revealed good properties as an affinity membranes and will be effective in processing large volumes of liquid culture medium containing a target protein.

References

[1] J.F. Kenedy, M. Paterson, Polymer Int. 32 (1993) 71.
[2] N. Labrou, Y.D. Clonis, J. Biotechnol. 36 (1994) 95.
[3] D. Guoqiang, R. Kaul, B. Mattiasson, J. Chromatogr. A 668 (1994) 145.
[4] H.J. Park, C.O. Reiser, S. Kondruweit, H. Erdmann, R.D. Schmid, M. Sprinzl, Eur. J. Biochem. 205 (1992) 881.
[5] T. Prestera, H.J. Prochaska, P. Talalay, Biochemistry 31 (1992) 824.
[6] N. Kubota, Y. Nakagawa, Y. Eguchi, J. Appl. Polym. Sci. 62 (1996) 1153.
[7] A. Denizli, G. Köktürk, B. Salih, A. Kozluca, E. Pişkin, J. Appl. Polym. Sci. 63 (1997) 27.
[8] J. Porath, J. Carlsson, I. Olsson, G. Belfage, Nature 258 (1975) 598.
[9] D.C. Rijken, G. Wijngssrds, M.Z. Jong, Biochim. Biophys. Acta 580 (1979) 140.
[10] D.C. Rijken, D. Collen, J. Biol. Chem. 256 (1981) 7035.

[11] I. Ohkubo, T. Kondo, N. Tanoguchi, Biochim. Biophys. Acta 616 (1980) 89.
[12] B. Lonnerdal, J. Carlsson, J. Porath, FEBS Lett. 75 (1977) 89.
[13] C.A. Berrebaeck, B. Lonnerdal, M.Z. Etzler, FEBS Lett. 130 (1981) 194.
[14] K.J. Chedha, P.M. Grob, A.J. Mikulski, Gen. Virol. 43 (1979) 701.
[15] M. Marquez-Mendez, J. Biochem. Biophys. Methods 24 (1992) 51.
[16] W. Guo, Z. Shang, Y. Yu, L. Zhou, J. Chromatogr. A 685 (1994) 344.
[17] N. Kubota, S. Miura, K. Saito, K. Sugita, K. Watanabe, T. Sugo, J. Membrane Sci. 117 (1996) 135.
[18] C.H. Bamford, G. Al-Lamee, M.D. Purbrick, T.J. Wear, J. Chromatogr. 606 (1992) 19.
[19] M. Kim, K. Saito, S. Furusaki, T. Sugo, I. Ishigaki, J. Chromatogr. 586 (1991) 27.
[20] F.T. Sarfert, M.R. Etzel, J. Chromatogr. A 764 (1997) 3.
[21] P. Langlotz, K.H. Kroner, J. Chromatogr. 591 (1992) 107.
[22] M.Y. Arıca, V. Hasırcı, J. Chem. Tech. Biotechnol. 58 (1993) 287.
[23] A. Denizli, B. Salih, M.Y. Arıca, K. Kesenci, V. Hasırcı, E. Pişkin, J. Chromatogr. A 758 (1997) 217.
[24] M.Y. Arıca, V. Hasırcı, Biomaterials 14 (1993) 803.
[25] M.Y. Arıca, V. Hasırcı, Biomaterials 14 (1993) 809.
[26] M.Y. Arıca, V. Hasırcı, Polymer Int. 32 (1993) 177.
[27] İ. Gürsel, M.Y. Arıca, C. Balcık, O. Akkuş, N. Akkaş, V. Hasırcı, Eng. Systems Design Anal. 5 (1996) 107.
[28] A. Denizli, B. Salih, A. Kozluca, E. Pişkin, J. Biomater. Sci. Polymer Ed. 8 (1997) 411.
[29] I.Y. Galaev, N. Garg, B. Mattiasson, J. Chromatogr. A 684 (1994) 45.
[30] J. Kirchberger, H. Erdmann, H.-J. Hecht, G. Kopperschlager, J. Chromatogr. A 668 (1994) 153.
[31] V. Radjendirane, M.A. Bhat, C.S. Vaidyanathan, Arch. Biochem. Biophys. 288 (1991) 169.
[32] H.J. Prochaska, Arch. Biochem. Biophys. 267 (1988) 529.
[33] C.M. Yea, A.R. Cross, O.T. Jones, Biochem. J. 265 (1990) 95.
[34] Q. Ma, R. Wang, C.S. Yang, A.Y. Lu, Arch. Biochem. Biophys. 283 (1990) 311.
[35] K.K. Kim, D.R. Fravel, G.C. Papavizas, Can. J. Microbial. 36 (1990) 760.
[36] G. Gunzer, F. Hendrick, J. Chromatogr. 296 (1984) 221.
[37] N.E. Labrou, A. Karagouni, Y.D. Clonis, Biotechnol. Bioeng. 48 (1995) 278.
[38] A. Tuncel, A. Denizli, D. Purvis, C.R. Lowe, E. Pişkin, J. Chromatogr. 634 (1993) 161.
[39] X. Zeng, E. Ruckenstein, J. Membrane Sci. 117 (1996) 271.