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Human serum albumin chromatography by Cibacron Blue F3GA-derived microporous polyamide hollow-fiber affinity membranes

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Abstract

An affinity dye ligand, Cibacron Blue F3GA was covalently attached onto commercially available microporous polyamide hollow-fibre membranes for human serum albumin (HSA) adsorption from both aqueous solutions and human plasma. Different amounts of Cibacron Blue F3GA were incorporated on the polyamide hollow-fibres by changing the dye attachment conditions, i.e. initial dye concentration, addition of sodium carbonate and sodium chloride. The maximum amount of Cibacron Blue F3GA attachment was obtained at 42.5 μmol g^{-1} when the hollow-fibres were treated with 3 *M* HCl for 30 min before performing the dye attachment. HSA adsorption onto unmodified and Cibacron Blue F3GA-derived polyamide hollow-fibre membranes was investigated batchwise. The non-specific adsorption of HSA was very low (6.0 mg g^{-1} hollow-fibre). Cibacron Blue F3GA attachment onto the hollow-fibres significantly increased the HSA adsorption (147 mg g^{-1} hollow-fibre). The maximum HSA adsorption was observed at pH 5.0. Higher HSA adsorption was observed from human plasma (230 mg HSA g^{-1} hollow-fibre). Desorption of HSA from Cibacron Blue F3GA derived hollow-fibres was obtained using 0.1 *M* Tris–HCl buffer containing 0.5 *M* NaSCN or 1.0 *M* NaCl. High desorption ratios (up to 98% of the adsorbed HSA) were observed. It was possible to reuse Cibacron Blue F3GA derived polyamide hollow-fibre without significant decreases in the adsorption capacities.

Keywords: Polyamide hollow-fibre; Affinity membranes; Human serum albumin; Cibacron blue F3GA

1. Introduction

Human serum albumin is the most abundant protein in blood plasma. It has many important physiological functions which contribute significantly to colloid osmotic blood pressure and aid in the transport, distribution and metabolism of many endogeneous and exogeneous substances including bile acids, bilirubin, long-chain fatty acids, amino acids (notably tryptophan, thyroxine and cysteine), steroids (progesterone, testosterone, aldosterone, cortisol), metal ions such as copper, zinc, calcium, magnesium, chloride and numerous pharmaceuticals [1]. Human serum albumin consists of a single, non-glycosylated, polypeptide chain containing 585 amino acid residues. Its amino-acid sequence contains a total of 17 disulphide bridges, one free thiol and a single tryptophan [2]. Research on albumin separation has attracted considerable attention for its great potential in blood protein manufacture. Human serum albumin is at present commonly isolated from human plasma by Cohn's classical blood fractiona-

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tion procedure [3]. Cohn's method concerns precipitation of proteins using ethanol with varying pH, ionic strength and temperature. But this technique, which is the oldest method of industrial fractionation of blood proteins, is not highly specific and can give partially denaturated proteins [4]. The biomimetic dye-ligands (i.e. triazine dyes) offer advantages over biological ligands in terms of economy, ease of immobilization, stability and high adsorption capacity [5]. Cibacron Blue F3GA is a group specific affinity dye that possess a high affinity to albumin [6].

In recent years, microporous membranes were modified and various affinity ligands were coupled for use as alternative supports for protein chromatography [7]. Microporous membranes have the advantages of large surface area, short diffusion path and low pressure drop. As a result of the convective flow of solution through the pores, the mass transfer resistance is tremendously reduced and the binding kinetics dominates the adsorption process. This results in a rapid processing, which greatly improves the adsorption, washing, desorption and regeneration steps and decreases the probability of inactivation of biomolecules [7]. The choice of the membrane material may be difficult as a compromise must be found regarding the reactivity of the material, stability in polar solvents, pore size and biocompatibility [8]. An ideal membrane for protein separation must fulfill the requirements of high hydrophilicity and low non-specific adsorption, fairly large pore size and a narrow pore size distribution, chemical and mechanical resistance as well as having enough reactive functional groups [9]. Nylon membranes offer narrow pore size distribution but, because of a low concentration of primary amino functional groups available in their structure, they have too low ligand density. These problems could be solved by hydration and binding with a polyhydroxyl-containing materials [10].

Recently, we prepared polyhydroxyehylmeth acrylate (PHEMA) based affinity sorbents and several bio-ligands (e.g. fibronectin, protein A, DNA, heparin) and dye-ligands (e.g., Cibacron Blue F3GA) were incorporated onto these bioaffinity sorbents for removal and separation of several substances (e.g., proteins, pathogenic antibodies, cholesterol, bilirubin, toxic metal ions such as cadmium(II)) from aqueous media including plasma in experimental batch set-up [11–17]. In this study, we attempted to prepare Cibacron Blue F3GA-attached polyamide hollow-fibre membranes as specific dye affinity sorbents for albumin adsorption from aqueous solutions and human plasma.

2. Experimental

2.1. Materials

Microporous polyamide hollow-fibre membranes (PA386C) were a gift from Akzo (Wuppertal, Germany). Cibacron Blue F3GA was obtained from Polyscience (Warrington, USA) and used without further purification. Human serum albumin (HSA, 98% pure by gel electrophoresis, fatty acid free, 67 kDa) was purchased from Aldrich (Munich, Germany). Acetic acid and miscellaneous chemicals were of analytical reagent grade and supplied from Merck (Darmstadt, Germany).Coomassie Blue for the Bradford Protein assay was from BioRad (Richmond, CA, USA). All water used in the experiments was purified using a Barnstead (Dubuque, IA) RO-pure LP® reverse osmosis unit with a high flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion-exchange packed bed system. The resulting purified water (deionized water) has a specific conductivity of 18 $M\Omega\,cm^{-1}$.

2.2. Cibacron blue F3GA-derived membranes

Polyamide hollow-fibres were used as received and cut to small segments (1 cm in length). Table 1

Table 1
Physical properties of the microporous polyamide capillary hollow fibre membrane

Type	Normal pore size (μm)	Max. pore size (μm)	BET surface area ($m^2\,g^{-1}$)	Wall thickness (μm)	Flux ($ml\,min^{-1}\,bar^{-1}\,cm^{-2}$)
P A 386 C	0.20	0.43	16.0	110	13

summarizes the physical properties of polyamide hollow-fibre membranes we used in this study as obtained from the manufacturer. The cut fibres were magnetically stirred (at 400 rpm) in a sealed reactor in 100 ml aqueous solution containing 300 mg Cibacron Blue F3GA for 30 min at 60°C. This was followed by the addition of 7.0 g NaCI in order to stimulate the deposition of the dye on the surface of the hollow fibre. After 30 min, 1.0 g sodium carbonate (Na_2CO_3) was added to accelerate the reaction between dye and hollow-fibre at 80°C for 4 h. In order to change the amount of Cibacron Blue F3GA incorporated to polyamide hollow-fibre, the initial concentration of Cibacron Blue F3GA was varied between 0.1 and 0.6 mg ml^{-1}. After incubation, the Cibacron Blue F3GA-derived polyamide hollow-fibres were washed with distilled water and methanol several times until all the physically attached Cibacron Blue F3GA molecules were removed. The modified hollow fibres were then stored at 4°C with 0.02% sodium azide to prevent microbial contamination.

In other dyeing preparations, in order to optimize the amount of dye incorporated into the polyamide hollow-fibres, these materials were exposed to partial hydrolysis under condition of not destroying mechanical integrity [18,19] as follows: The hollow fibres were magnetically stirred at 100 rpm with 3 *M* HCI at a constant temperature of 30°C for 20 min. The acid hydrolysis was then arrested by washing with cold water (4°C). After hydrolysis the polyamide hollow-fibre membranes were dyed with Cibacron Blue F3GA applying the optimal parameters determined from the previous preparations using the same method mentioned above.

2.3. Characterisation of hollow-fiber membranes

2.3.1. Elemental analysis

The amount of Cibacron Blue F3GA immobilized on the hollow-fibre was evaluated by using an elemental analysis instrument (Leco, CHNS-932 USA) by considering the sulfur stoichiometry.

2.3.2. SEM studies

In order to observe the surface and bulk structures of the polyamide hollow-fibres, scanning electron micrographs of coated samples were taken with a SEM (Model: Raster Electronen Microscopy, Leitz-AMR-1000, Germany).

2.4. HSA adsorption from aqueous solutions

In these experiments, the effects of initial protein concentration, medium pH, amount of Cibacron Blue F3GA attached and ionic strength on the adsorption capacity of Cibacron Blue F3GA derived polyamide hollow fibre were studied. The adsorption experiments were carried out batchwise in the media at different pH values. The pH of the adsorption medium was varied between 4.0 and 8.0 using different buffer systems (0.1 *M* CH_3COONa–CH_3COOH for pH 4.0–6.0, 0.1 *M* K_2HPO_4–KH_2PO_4, for pH 7.0 and 0.1 *M* Tris–HCl for pH 8.0). Ionic strength of the adsorption media was changed in the range of 0.05–0.5. Human serum albumin concentration was varied between 1.0 and 7.0 mg ml^{-1}. The amount of Cibacron Blue F3GA attached on the hollow fibre was varied between 14.7 and 42.5 μmol g^{-1}. In a typical adsorption experiment, HSA was dissolved in 10 ml of buffer solution, and 50 pieces of hollow-fibres (total length: 50 cm) were added. Then the adsorption experiments were conducted for 2 h (equilibrium time) at 4°C at a stirring rate of 100 rpm. At the end of this equilibrium period, HSA adsorption by the Cibacron Blue F3GA-derived hollow fibre was determined by measuring the initial and final concentration of HSA within the adsorption medium using Coomassie Brilliant Blue as described by Bradford [20].

2.5. HSA adsorption from human plasma

Human serum albumin adsorption from human plasma with Cibacron Blue F3GA-derived polyamide hollow-fibre membranes was studied batch-wise. The blood was obtained from a healthy human donor. Blood samples were centrifuged at 500 g for 3 min at room temperature to separate the plasma. The original plasma of the donor contained 41.0 mg HSA ml^{-1} as determined by bromocresol green (BCG) dye method at 628 nm [21]. 10 ml of the freshly separated human plasma was incubated with 50 hollow fibre cuts (total length: 50 cm) pre-equilibrated with acetate buffer (pH 5.0) for 2 h. These experiments were conducted at 4°C and a stirring rate of 100 rpm. The amount of HSA adsorbed by

Cibacron Blue F3GA-hollow-fibres was determined by measuring the initial and final concentration of HSA in plasma. Phosphate buffered saline (PBS, pH: 7.4, NaCl: 0.9%) was used for dilution of human plasma.

In order to show dye specificity, competitive blood protein adsorption (i.e., albumin, fibrinogen and immunoglobulin-G) was also studied. The dye-attached hollow fiber membranes were incubated with a human plasma containing albumin (41.0 mg ml^{-1}), fibrinogen (2.4 mg ml^{-1}) and γ-globulin (18.2 mg ml^{-1}) at room temperature for 2 h. Total protein concentration was measured by using the total protein reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, UK; Catalog Ref. No: 712076) at 540 nm which based on Biuret reaction [21]. Chronometric determination of fibrinogen according to the Clauss method on plasma was performed by using Fibrinogene-Kit (Ref. No: 68452 and 68582, bioMerieux Laboratory Reagents and Instruments, Marcy-l'Etoile, France) [22]. Human serum albumin concentration was determined by using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd, Halstead, Essex, UK; Catalog Ref. No: 229241) which based on bromocresol green (BCG) dye method [21]. γ-globulin concentration was determined from the difference.

2.6. Desorption studies

The desorption of HSA was carried out using 0.05 Tris–HCl buffer containing 0.5 *M* NaSCN or 1.0 *M* NaCl, (pH 8.0) at room temperature. The HSA adsorbed HF (50 pieces) were placed in the desorption medium and stirred for 1 h, at 25°C, at a stirring rate of 100 rpm. The final HSA concentration within the desorption medium was determined by using Coomassie Brilliant Blue as described by Bradford.

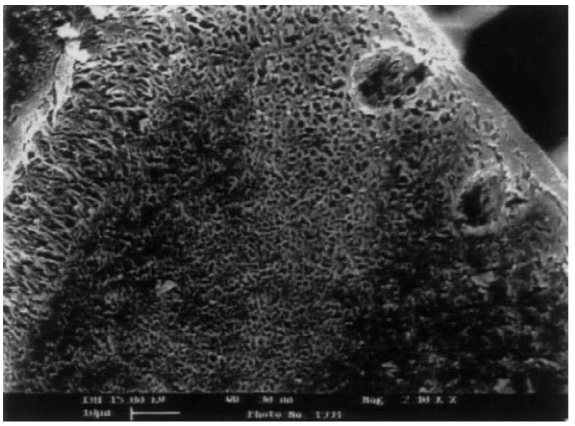
3. Results and discussion

3.1. Characteristics of polyamide hollow-fiber membranes

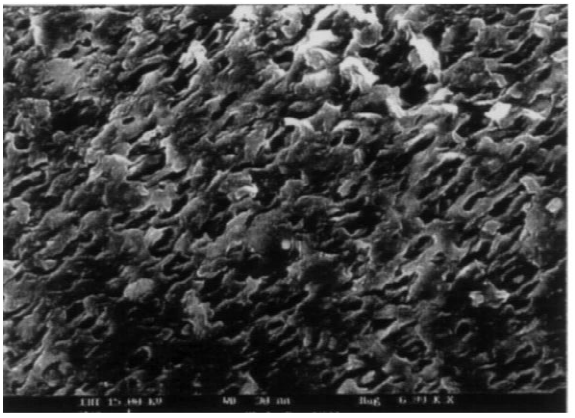
An ideal membrane for protein chromatography must have the following properties; high hydrophilicity and low non-specific adsorption, fairly large pore size and a narrow pore size distribution, chemical and mechanical resistance as well as enough reactive functional groups. Polyamide hollow-fibre membrane may meet most of these requirements, since they have a narrow pore size distribution and good mechanical rigidity. The scanning electron microscope (SEM) micrographs given in Fig. 1 show the surface structure and the cross-section of the polyamide hollow-fibre membranes. As seen in these micrographs, the smallest pore structure of the fibre was highly asymmetric. Furthermore, the smallest pores occurred at the lumen side of the fibre while the pore size at the shell side was much larger. However, polyamide hollow-fibre membranes have several disadvantages. They have a low concentration of primary amino groups leading to low ligand density and a non-specific adsorption of protein. These problems can be solved by hydrating the membranes to increase the number of primary amino groups on the membrane, binding with polyhydroxyl-containing materials such as poly-glucose, dextran, starch and cellulose to increase reactive sites and reduce non-specific protein adsorption.

Cibacron Blue F3GA was used as the dye-ligand for specific binding of human serum albumin molecules. Fig. 2 gives the chemical structure of this dye. Note that the possible active sites to react with proteins are SO_3H, NH_2, NH. It is reported that Cibacron Blue F3GA has no side effect on biochemical systems [23]. However, all commercial reactive dyes (including Cibacron Blue F3GA) contain various impurities which may affect their biochemical and related use. Reactive dyes have been purified by a number of chromatographic procedures such as thin layer chromatography, high-performance liquid chromatography and column chromatography on Silica gel or sephadex. However it is suggested that purification of reactive dyes is necessary only when free dyes are used [24]. In cases where attached dyes are used, purification of the dye before immobilization is not likely to be necessary, because few of the contaminants will be attached on the support matrix, and proper washing of the matrix should remove adsorbed contaminants [25].

In this study, Cibacron Blue F3GA is covalently attached on polyamide hollow-fibre membranes, via the reaction between the chloride groups of the reactive dyes and the primary amino groups of the



(a)



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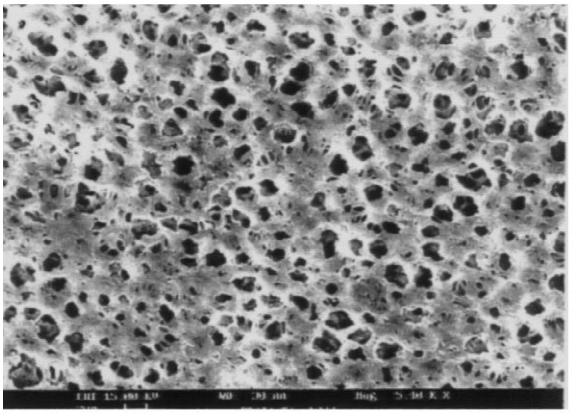

(c)

Fig. 1. Representative SEM micrographs of polyamide hollow-fibre membranes. (a) Inner surface; (b) outer surface; (c) cross-section.

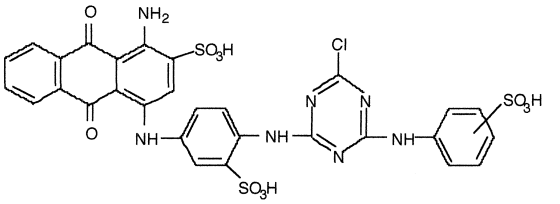

Fig. 2. Chemical structure of Cibacron Blue F3GA.

polyamide membranes. The coupling of Cibacron Blue F3GA to polyamide hollow-fibre membranes was studied under different conditions. The dye densities reached on polyamide membranes using different parameters are listed in Table 2. The highest dye surface density obtained without hydrating of polyamide membranes was (35.8 μmol g^{-1}). When polyamide hollow-fibre membranes hydrating with HCl the amount of Cibacron Blue F3GA was much higher (42.5 μmol g^{-1}). The higher dye-ligand densities are result from the larger number of primary amino groups produced as a result of the acid hydration.

The studies of Cibacron Blue F3GA leakage from the polyamide hollow-fibre membranes showed that there was no dye leakage in any medium used throughout this study, even in long period of time (more than 8 weeks).

3.2. HSA adsorption from aqueous solutions

Cibacron Blue F3GA has been found to interact with many proteins that possess a dinucleotide-binding domain [26]. However, other proteins such as interferon [27] and human serum albumin [28] which may not possess a dinucleotide-domain have also

Table 2
Influence of Cibacron Blue F3GA concentration, and HCl treatment on the dye attachment

Cibacron Blue F3GA Concentration (mg ml^{-1})	Cibacron Blue F3GA[a] Loading (μmol g^{-1})
0.1	14.7±0.89
0.2	23.5±0.76
0.3	29.6±0.81
0.4	33.9±0.91
0.6	35.8±0.79
0.3 (HCl treatment)	42.5±0.68

[a] Each data is average of three parallel studies.

been shown to bind to Cibacron Blue F3GA. Furthermore, it is proposed that the mechanism(s) of interaction between HSA and Cibacron Blue F3GA are consistent with Cibacron Blue F3GA binding to bilirubin-binding sites [28]. Various experimental factors can effect the interactions including pH, HSA initial concentration, ionic strength and dye-ligand surface density.

3.3. Influence of dye loading on HSA adsorption

In order to study the effect of dye concentration on the HSA adsorption, poly amide hollow-fibre with different densities were evaluated. Fig. 3 provides the influence of dye loading on the HSA adsorption. The highest protein adsorbed was observed in the case with the highest dye loading (147 mg HSA g^{-1} hollow-fibre). This may be explained as follows, when the dye loading increases the attached amount of negative charge on the membrane surface which will interact with the HSA will increase leading to higher proteins adsorption.

3.4. Effects of pH

Fig. 4 reveals the effect of pH on the adsorption of HSA onto Cibacron Blue F3GA-derived polyamide hollow-fibre membranes. In all the investigated cases, the maximum adsorption of HSA was observed at pH 5.0, which is the isoelectric point of HSA. With the increase of pH above and below the pH 5.0, the HSA adsorption capacity decreased. The decrease in the HSA adsorption capacity can be attributed to electrostatic repulsion effects between the identically charged groups. At the isoelectric points, proteins have no net charge and therefore, the maximum adsorption from aqueous solution is usually observed at these points [29]. In addition, these interactions between the dye and protein molecules may result both from the ionization states of several groups on both the ligands (i.e., Cibacron Blue F3GA) and amino acid side chains in human serum albumin structure, and from the conformational state of protein molecules at this pH [29]. It should be also noted that non-specific adsorption is independent of pH and it is observed at the same at all the pH values studied.

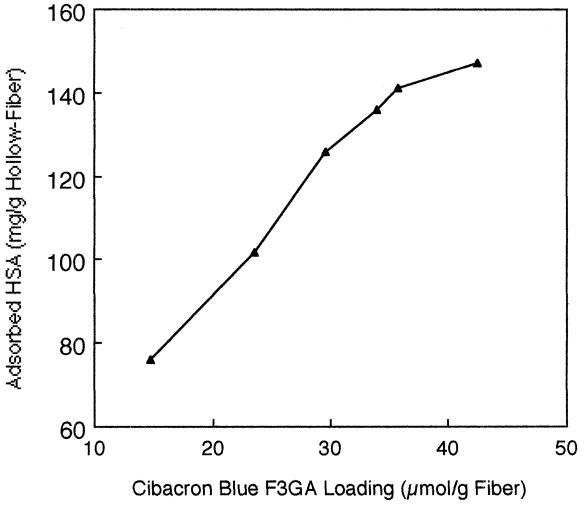

Fig. 3. Effect of the Cibacron Blue F3GA loading on the HSA adsorption. Initial HSA concentration: 5.0 mg ml^{-1}; pH: 5.0; and temperature: 4°C. Each data is average of five parallel studies.

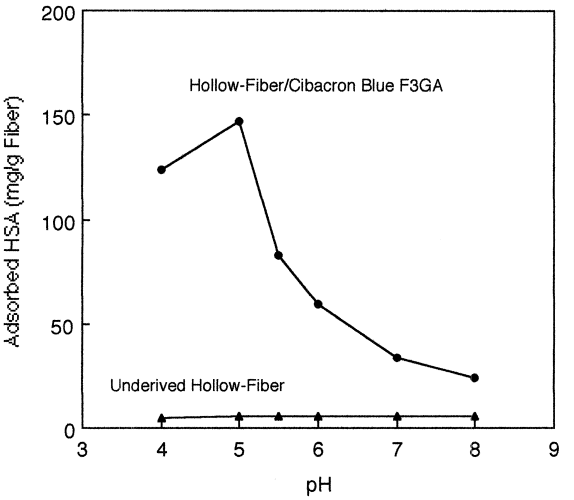

Fig. 4. Effect of medium pH on the HSA adsorption on Cibacron Blue F3GA-attached polyamide hollow-fibre: Cibacron Blue F3GA loading: 42.5 μmol g^{-1}; HSA initial concentration: 5 mg ml^{-1}; temperature: 4°C. Each data is average of five parallel studies.

3.5. Effect of HSA initial concentration

Fig. 5 shows the effects of initial concentration of HSA on the amount of HSA adsorbed. As seen in this figure, with increasing HSA concentration in

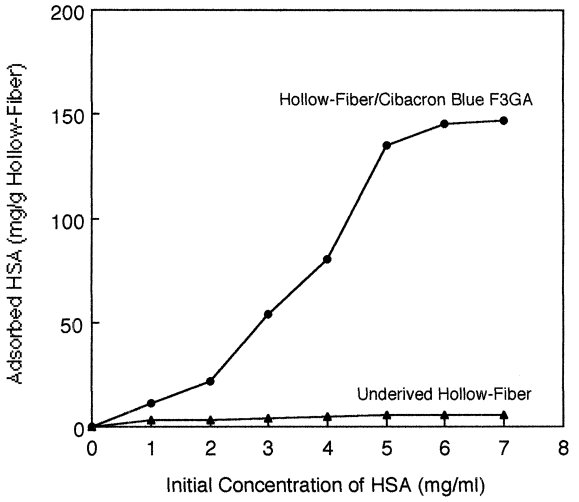

Fig. 5. Effect of the initial concentration of HSA on the Cibacron Blue F3GA-attached polyamide hollow fibre: Cibacron Blue F3GA loading: 42.5 μmol g^{-1}; pH: 5.0; and temperature: 4°C. Each data is average of five parallel studies.

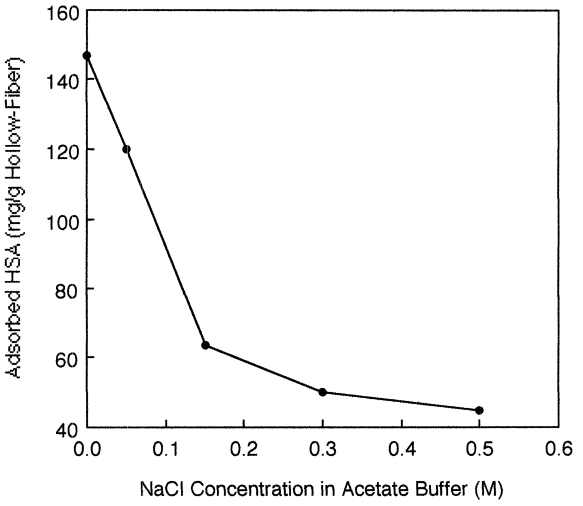

Fig. 6. Effect of the Ionic strength on HSA adsorption on the Cibacron Blue F3GA-attached polyamide hollow-fibre membranes; Cibacron Blue loading: 42.5 μmol g^{-1}; initial HSA: 5.0 mg ml^{-1}; pH:5.0, and temperature: 4°C. Each data is average of five parallel studies.

solution, the adsorbed amount of HSA per unit mass of hollow-fibre increases until about 5.0 mg ml^{-1}, then approaches saturation. Negligible amounts of HSA adsorbed non-specifically on the plain polyamide hollow-fibre membrane (6.0 mg g^{-1}). Cibacron Blue F3GA attachment significantly increased the HSA adsorption capacity of the hollow-fibre membranes (up to 147 mg HSA g^{-1}). It is clear that this increase in adsorption capacity is due to specific interaction between Cibacron Blue F3GA and HSA molecules which promote the adsorption of albumin.

3.6. Effect of ionic strength

The effect of ionic strength (adjusted by adding NaCl) on HSA adsorption is presented in Fig. 6, which shows that the adsorption capacity decreases with increasing ionic strength of the binding buffer (acetate buffer, pH: 5.0). The adsorption of HSA decreases by about 67% as the NaCl concentration changes from 0.05 to 0.5 *M*. The decrease in the adsorption capacity as the ionic strength increases can be attributed to the repulsive electrostatic interactions between the dye-attached hollow-fibre membranes and protein molecules. When the salt concentration increases in the adsorption medium, this can lead to coordination of the deprotonated sulfonic acid groups of the dye with sodium ions of the salt (NaCl), which leads to low protein adsorption. The distortion of existing salt bridges in the presence of salt also contributed to this low protein adsorption at high ionic strength.

3.7. HSA adsorption from human plasma

The adsorption of HSA from human plasma was performed batch wise. Table 3 shows the adsorption for human serum obtained from a healthy donor. There was a very low non-specific adsorption of HSA (7.5 mg g^{-1}) on the untreated polyamide hollow-fibre membranes, while much higher adsorp-

Table 3
HSA adsorption from the plasma of a healthy donor[a]

HSA concentration (mg ml^{-1})	Amount of HSA adsorbed[b] (mg g^{-1})
10.3	120.1±2.2
20.5	156.5±3.2
30.8	168.4±3.0
41.0	230.0±2.9

[a] Ligand surface concentration: 42.5 μmol g^{-1}; temperature: 20°C; plasma volume: 4.0 ml.

[b] Each data is average of five parallel studies.

tion values (230 mg g^{-1}) were obtained when the Cibacron Blue F3GA-attached hollow-fibre membranes were used. It is worth to note that adsorption of HSA onto the Cibacron Blue F3GA-attached hollow-fibre membranes was approximately 1.6 fold higher than those obtained in the studies in which aqueous solutions were used. This may be explained as follows; the conformational structure of HSA molecule within their native environment (i.e. human plasma) much more suitable for specific interaction with the Cibacron Blue F3GA-hollow-fibre membrane. The high HSA concentration (41.0 mg ml^{-1}) may also contribute to this high adsorption due to the high driving force between the aqueous (i.e., human plasma) and solid-phases (i.e., hollow-fibre membrane) [28].

In order to show the dye specificity, competitive protein adsorption was also studied. Interesting results were obtained in these studies. Adsorption capacities were obtained as 230 mg g^{-1} for albumin, 5.8 mg g^{-1} for fibrinogen and 12.5 mg g^{-1} for γ-globulin. The total protein adsorption was determined as 252.1 mg g^{-1}. It is worth noting that adsorption of other plasma proteins (i.e., fibrinogen and gamma-globulin) on the Cibacron Blue F3GA-derived hollow-fiber membranes are negligible. It should be noted that albumin is the most abundant protein in plasma. It generally makes up more than half of the total plasma proteins. It may be resulted that this low adsorption of fibrinogen and gamma-globulin is due to the high concentration of albumin.

3.8. Comparison with related literature

Different dye-affinity sorbents with different adsorption capacities were reported in literature for albumin adsorption. Nigel et al. used dye-incorporated Sepharose CL-6B-200 and they reported bovine serum albumin (BSA) adsorption capacities around 1–3 mg g^{-1} moist gel [29]. Denizli et al. used different dye and metal-chelate affinity polymeric sorbents including monosize poly(methylmethacrylate-hydroxyethylmethacrylate), poly(vinylalcohol) and poly(hydroxy-ethylmethacrylate) microspheres and they obtained 35–178 mg g^{-1} polymer for bovine and human serum albumin [30–32].Nash and Chase used poly(vinyl alcohol) modified poly-(styrene-divinyl benzene) microbeads carrying different dye ligands [33]. They presented adsorption capacities of 11.7–27 mg HSA g^{-1}. Boyer and Hsu used Sepharose beads carrying different amounts of Cibacron Blue F3GA (2–25 μmol ml^{-1}) and reported adsorption values up to 55.9 mg BSA per g polymer [34]. Zeng and Ruckenstein reported 10.2 mg HSA g^{-1} adsorption capacity with Cibacron Blue F3GA-attached-polyethersulfone supported chitosan sorbents [35]. Li and Spencer used Cibacron Blue F3GA-attached polyethylene imine coated titania and achieved 4.4 mg HSA g^{-1} [36]. Chase reached 14 mg BSA g^{-1} with Cibacron Blue F3GA-attached Sepharose CL-6B [37]. Tuncel et al. reported 60 mg BSA g^{-1} polymer with Cibacron Blue F3GA-attached poly(vinyl-alcohol)-coated monosize polystyrene microbeads [38]. Muller-Shulte et al. used several polymeric carriers made of different polymers, and Cibacron Blue F3GA as the dye-ligand [39]. Their albumin adsorption values were in the range of 0.19–0.81 mg HSA ml^{-1} sorbent. Adsorption capacities of commercially available cross-linked agarose/Cibacron Blue F3GA sorbents (Bio-rad, California, USA) were reported as about 11 mg albumin per milliliter sorbent [40]. Comparison of these results shows that Cibacron Blue F3GA-attached polyamide hollow-fibre membranes exhibit much higher human serum albumin adsorption capacities.

3.9. Desorption studies

The desorption experiments were performed using eluents,1.0 *M* NaCl or 0.5 *M* NaSCN in 0.01 *M* Tris–HCl buffer at pH 8.0. The desorptions of human serum albumin are expressed in % of totally adsorbed HSA:

The results showed that about 78% of the adsorbed human serum albumin molecules were eluted with 1.0 *M* NaCl eluents. However, about 98% of human serum albumin could be desorbed with 0.5 *M* NaSCN eluent. These observations can be explained depending on the electrostatic attraction between the positively charged domains of the protein and the negatively charged $-SO_3$ of the Cibacron Blue F3GA when the ionic strength increases by NaCl and NaSCN addition the electrostatic attraction men-

tioned previously will be decreased and this will stimulate the HSA desorption. The much stronger desorption which arises in the presence of salt may be attributed to the disorganisation of the structure of water by NaSCN [24]. This is an important feature indicating the possibility of recycling hollow fibers for reuse in the protein separation process.

4. Conclusions

Polymer membranes with well-defined pore structures and specifically functionalized surfaces play an important role in advanced separation, e.g., high-performance ultrafiltration fractionation [41] or affinity filtration of proteins [42]. Affinity membrane sorption is a developing bioseparation technique that has been studied for various proteins such as urokinase [43], avidin [44] and immunoglobulin G [45,46]. A commercially available microporous polyamide hollow-fibre membrane with covalently attached synthetic dye-ligand (i.e., Cibacron Blue F3GA) was developed for affinity filtration of human serum albumin (HSA). The results obtained in this communication show that adsorption of HSA under different pH values, ionic strength, dye-ligand surface densities, and HSA initial concentration have revealed the high selectivity and affinity of Cibacron Blue F3GA polyamide hollow-fibre for HSA. It was possible to use the Cibacron Blue F3GA-attached microporous polyamide hollow-fibre membranes in a HSA adsorption-desorption cycle.

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