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BILIRUBIN REMOVAL FROM HUMAN PLASMA BY DYE AFFINITY MICROPOROUS HOLLOW FIBERS

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ABSTRACT

Bioaffinity adsorption has a unique and powerful role as a support tool in the removal of toxic substances from human plasma. Synthetic hollow-fiber membranes have advantages as support matrices in comparison to conventional hemoperfusion columns because they are not compressible and they eliminate internal diffusion limitations. In this study, Cibacron Blue F3GA was covalently attached onto commercially available microporous polyamide hollow-fiber membranes for bilirubin removal from hyperbilirubinemic human plasma. Different amounts of Cibacron Blue F3GA were attached on the polyamide hollow-fibers by changing the dye-attachment conditions, i.e., initial dye concentration, addition of sodium carbonate, and sodium chloride.

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The maximum amount of Cibacron Blue F3GA attachment was obtained at 42.5 μmol g^{-1} when the hollow fibers were treated with 3 *M* HCl for 30 min before performing the dye attachment. The nonspecific bilirubin adsorption on the unmodified polyamide hollow-fiber membranes was 0.65 mg g^{-1} from human plasma. Higher bilirubin adsorption capacities, of up to 39.7 mg g^{-1}, were obtained with the Cibacron Blue F3GA-attached polyamide hollow-fiber membranes. Further increase in bilirubin adsorption was obtained as 48.9 mg g^{-1}. Bilirubin molecules interacted with these adsorbents directly. Contribution of albumin adsorption on the bilirubin adsorption was much pronounced. Bilirubin adsorption increased with increasing temperature and maximum adsorption was observed at 37°C.

Key Words: Hyperbilirubinemia; Bilirubin removal; Cibacron Blue F3GA-attached polyamide hollow fibers

INTRODUCTION

Bilirubin is a metabolite of the heme from hemoglobin in the blood. The free bilirubin is toxic, and hence it is transported to the liver as a complex with albumin where it is normally conjugated and excreted into the bile (1). High bilirubin concentration may cause hepatic or biliary tract dysfunction, and also permanent brain damage or death in more severe cases (2). There have been many studies including removal of bilirubin directly from plasma of patients suffering from hyperbilirubinemia by hemoperfusion treatment, i.e., circulation of blood through an extracorporeal unit containing an adsorbent system for bilirubin (3–19). Activated charcoal (5) and agar (6) have been used as sorbents in hemoperfusion. In most cases, ion-exchange resins have been utilized (7,8). It has been shown that uncharged resins can adsorb bilirubin from aqueous media (9,10). Idezuki et al. have used anion-exchange synthetic fibers, and clinically applied this sorbent system to a selective bilirubin separation (11). Sideman et al. suggested the application of hemoperfusion to the removal of the bilirubin from jaundiced newborn babies by using albumin-deposited macroreticular resin (12). Brown prepared oligo-peptide functionalized polyacrylamide beads as affinity sorbent system for bilirubin removal (13). Chandy and Sharma used polylysine-carrying chitosan beads for selective bilirubin removal (14). Yamazaki et al. developed poly(styrene–divinyl benzene)-based sorbents, and successfully applied them in the treatment of more than 200 patients with hyperbilirubinemia (15). Morimoto et al. used plasma exchange and plasma adsorption with styrene–

divinyl benzene resin and removed bilirubin from hepatectomized patients. This plasma adsorption system provided a possibility for an improved supportive therapy for hepatic failure, especially for patients with hepatic coma and hyperbilirubinemia (16). Mor et al. conjugated human serum albumin with agarose using the cyanogen bromide and reported high bilirubin-binding capacity (17). Yu et al. synthesized amine-containing crosslinked chitosan resins and investigated adsorption behavior of conjugated bilirubin (18). Kuroda et al. studied selective adsorption of bilirubin by macroporous poly(glycidyl methacrylate-*co*-divinyl-benzene) beads (19).

In recent years, microporous membranes were modified and various affinity ligands were coupled for use as alternative adsorbents for biomedical applications (20,21). 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 step. 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 (22). An ideal membrane for biomedical application must fulfill the requirements of high hydrophilicity and low nonspecific protein adsorption, fairly large pore size and a narrow pore size distribution, chemical and mechanical resistance as well as having enough reactive functional groups (23). Nylon membranes offer narrow pore-size distribution, but because of low concentration of primary amino functional groups available in their structure, they have very low-ligand density. These problems could be solved by hydration and binding with polyhydroxyl-containing materials (24).

Different functional biomolecules, including enzymes, coenzymes, cofactors, antibodies, amino acids, oligopeptides, proteins, nucleic acids, and oligonucleotides may be used as ligands in the design of novel adsorbents (25). These ligands are extremely specific in most cases. However, they are expensive due to high cost of production and/or extensive purification steps. In the process of the preparation of specific sorbents, it is difficult to immobilize certain ligands on the supporting matrix with retention of their original biological activity. Precautions are also required in their use (at sorption and elution steps) and storage. Dye-ligands have been considered as one of the important alternatives to their natural counterparts for specific affinity chromatography to circumvent many of the above-mentioned drawbacks. Dye-ligands are able to bind most types of proteins, especially enzymes, in some cases in a remarkably specific manner (26). They are commercially available, inexpensive, and can be easily immobilized, especially on matrices-bearing hydroxyl groups. Although dyes are all synthetic in nature, they are still classified as affinity ligands

because they interact with the active sites of many biomolecules by mimicking the structure of the substrates, cofactors, or binding agents for those biological molecules (27).

Recently, our interest was focused on preparation of a hollow-fiber-based bioaffinity adsorbents. The authors used commercially available polyamide hollow-fiber membranes as the support. Cibacron Blue F3GA was covalently attached to the hollow fibers in alkaline medium. Bilirubin adsorption on the dye-attached hollow fibers was investigated from human plasma contain different amounts of bilirubin.

EXPERIMENTAL

Cibacron Blue F3GA-Attached Membranes

Microporous polyamide hollow-fiber membranes (PA386C) were a gift from Akzo (Wuppertal, Germany). Polyamide hollow-fibers were used as-received and cut into small segments (1 cm in length). Table 1 summarizes the physical properties of polyamide hollow-fiber membranes as obtained from the manufacturer. The cut fibers 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. Cibacron Blue F3GA was obtained from Polyscience (Warrington, USA) and used without further purification. This was followed by the addition of 7.0 g NaCl in order to stimulate the deposition of the dye on the surface of the hollow fiber. After 30 min, 1.0 g of sodium carbonate (Na_2CO_3) was added to accelerate the reaction between dye and hollow fiber at 80°C for 4 hr. In order to change the amount of Cibacron Blue F3GA attached to polyamide hollow-fiber, the initial concentration of Cibacron Blue F3GA was varied between 0.1 and 0.6 mg mL^{-1}. After incubation, the Cibacron Blue F3GA-attached polyamide hollow-fibers were washed with distilled water and methanol several times until all the physically attached Cibacron Blue F3GA molecules were removed. The modified hollow fibers 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 attached onto the polyamide hollow-fibers, these materials were exposed to partial hydrolysis under the condition of not destroying mechanical integrity as follows: the hollow fibers were magnetically stirred at 100 rpm with 3 *M* HCl 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-fiber membranes were dyed with Cibacron Blue F3GA applying the optimal parameters determined from the previous preparations using the same above-mentioned method.

Table 1. Physical Properties of the Microporous Polyamide Capillary Hollow-Fiber Membrane

Type	Normal Pore Size (μm)	Maximum Pore Size (μm)	BET Surface Area ($m^2\,g^{-1}$)	Wall Thickness (μm)	Flux ($mL\,min^{-1}\,bar^{-1}\,cm^{-2}$)	Inner Diameter (μm)	Outer Diameter (μm)
P A 386 C	0.20	0.43	16.0	110	13	300	410

Dye-Release Studies

In order to estimate the amount of released Cibacron blue F3GA, the hollow-fiber samples were placed in test tubes containing 10 mL of release media and shaken on a rotary shaker for 24 hr. The media were replaced with fresh media every 24 hr and the experiment was continued till no measurable release was observed. The amount of dye released into the medium was measured cumulatively as the absorption band at 630 nm for Cibacron Blue F3GA by a bench-top spectrophotometer (Spectronic-21 Series, Bousch and Lomb, Germany). Three kinds of release media were used: pH 2.0 buffer of acetic acid solution (50%, v/v), 0.1 *M* phosphate buffer solution (pH: 7.0), and 0.1 *M* sodium citrate/NaOH buffer solution (pH: 12.0).

Characterization of Hollow-Fiber Membranes

Elemental Analysis

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

Scanning Electron Microscopy Studies

In order to observe the surface and cross-section of the polyamide hollow-fibers, scanning electron micrographs of coated samples were taken with a Scanning Electron Microscopy (SEM) (Model: JEOL, JEM 1200 EX, Tokyo, Japan). Polyamide hollow fibers were dried at room temperature and coated with a thin layer of gold (about 100 Å) in vacuum and photographed in the electron microscope with $\times 800$ magnification.

Porosity Measurements

Pore volumes and average pore diameter greater than 20 Å were determined by mercury porosimeter up to 2000 kg cm^{-2} using a Carlo Erba model 200 (Milan, Italy). The surface area of the hollow-fiber sample was measured with a surface area apparatus (Brunner Emmet Teller, BET method).

Bilirubin Removal from Human Plasma

Bilirubin removal from human plasma with the unmodified and Cibacron Blue F3GA-attached polyamide hollow-fiber membranes was studied batch wise. The blood samples were obtained from the patients with hyperbilirubinemia. The plasma was separated by centrifugation at 500*g* for 30 min at room temperature. Since, bilirubin is destroyed by exposure to direct sunlight or any other source of ultraviolet light, including fluorescent lighting, all adsorption experiments were carried out in a dark room. Ten milliliters of the freshly separated human plasma was incubated with 50 hollow-fiber cuts (total length: 50 cm) at different temperatures (i.e., 4, 25, and 37°C) for 2 hr. Polyamide hollow-fiber membranes containing different amounts of Cibacron Blue F3GA on their surfaces were utilized. The amounts of bilirubin removed were determined by Malloy/Evelyn modified colorimetric test by measuring the decrease in the bilirubin concentration in the plasma samples (28). The amount of adsorbed bilirubin was calculated as:

$$q = [(C_i - C_t)\cdot V]/m \quad (1)$$

where, q is the amount of bilirubin adsorbed onto unit mass of the hollow-fiber (mg g^{-1}); C_i and C_t are the concentrations of the bilirubin in the initial and in the final plasma after adsorption, respectively (mg 100 mL^{-1}); V is the volume of the plasma (mL); and m is the mass of the hollow fiber (g).

Total protein concentration was measured using the total protein reagent (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK; Catalog Ref. No: 712076) at 540 nm, which is based on Biuret reaction (29). Human serum albumin concentration was determined using Ciba Corning Albumin Reagent (Ciba Corning Diagnostics Ltd., Halstead, Essex, UK; Catalog Ref. No: 229241), which was based on bromocresol green (BCG) dye method (29). Adsorption amounts for total protein and HSA were calculated using Eq. (1).

RESULTS AND DISCUSSION

Characteristics of Polyamide Hollow-Fiber Membranes

An ideal membrane for biomedical applications must have the following properties: high hydrophilicity and low nonspecific protein adsorption, fairly large pore size and a narrow pore size distribution, chemical and mechanical resistance as well as enough reactive functional groups. Polyamide hollow-fiber membranes may meet most of these requirements, since they have a narrow pore size distribution and good mechanical rigidity. According to the mercury

porosimetry data, the pore radii of the polyamide hollow-fiber membranes varied between 200 and 450 nm. This indicated that the hollow-fiber membranes contained mainly macropores. The SEM micrographs given in Fig. 1 show the surface structure and the cross-section of the polyamide hollow-fiber membranes. As seen in these micrographs, the smallest pore structure of the fiber was highly asymmetric. Furthermore, the smallest pores occurred at the lumen side of the fiber while the pore size at the shell side was much larger.

Specific surface area of the hollow-fiber membrane is found to be $16.0\,m^2\,g^{-1}$ polymer by BET method after Cibacron Blue F3GA attachment. Therefore these pores were not blocked by the dye molecules during the dye-attachment operation.

Cibacron Blue F3GA is covalently coupled to the polyamide hollow-fiber membranes via the nucleophilic reaction between the chloride of its triazine ring and the amine groups of the hollow fibers, under alkaline conditions (30). Note that although the amino acid sequence distribution of HSA is well documented, the precise location of the primary binding site for bilirubin has not been established yet. In our case, we also do not have any clear-cut evidence about the exact interaction points of bilirubin and Cibacron Blue F3GA molecules.

(a)

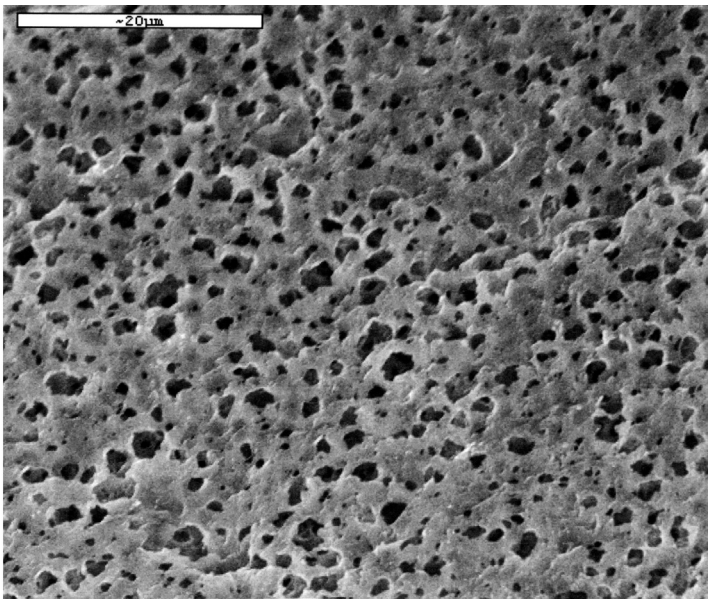
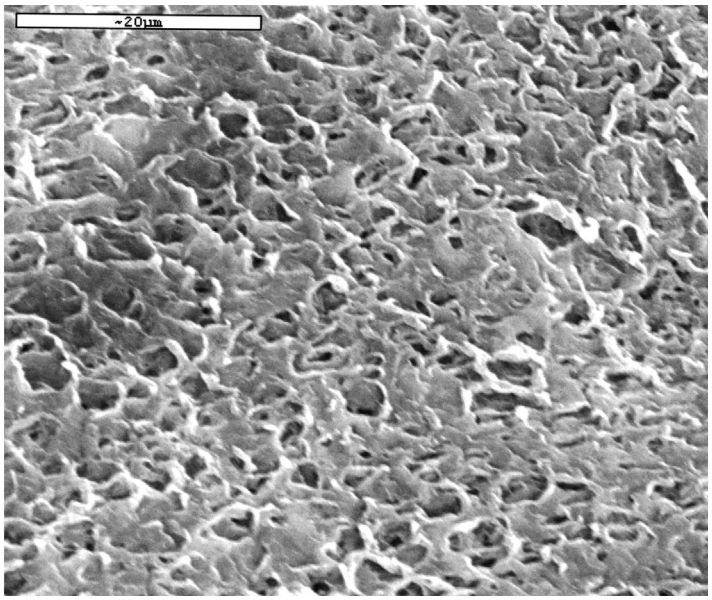


Figure 1. Representative SEM micrographs of polyamide hollow-fiber membranes: (a) inner surface; (b) outer surface; and (c) cross-section.

(b)



(c)

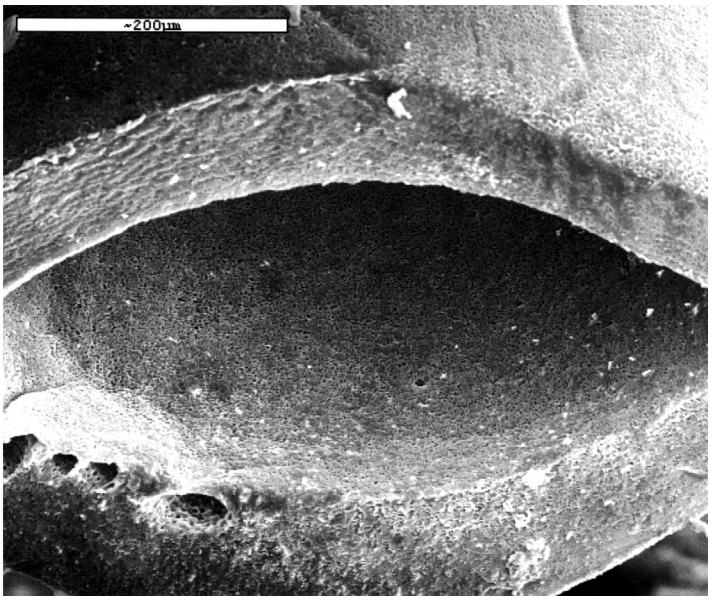


Figure 1. Continued.

However, from the chemical formulas of both molecules, we may postulate that these molecules may interact through the amino and carboxyl groups, which are the most probable reactions. Hydrophobic interactions between the aromatic groups in the dye structure and bilirubin molecules are also important.

Note that the release of dye molecules was measured in three different kinds of media. There was no measurable release of dye molecules into the acidic medium (pH 2.0). Dye was released in the neutral medium while some was released in the alkaline medium too (2%). The release in the strongly alkaline medium indicates the existence of strong ionic interactions. The release in the neutral medium (1%) might just be the physically occluded dye molecules along with any weakly/physically bonded dye. It can be said that there was not a significant increase in the amount of dye released.

Effects of Cibacron Blue F3GA Loading on Bilirubin Adsorption

In this group of experiments, we used human plasma samples obtained from a patient with hyperbilirubinemia, in which the total bilirubin concentration was 17.8 mg 100 mL^{-1}. The unmodified and Cibacron Blue F3GA-attached polyamide hollow-fiber membranes carrying different amounts of ligand (i.e., Cibacron Blue F3GA) were incubated with the plasma samples for 2 hr at room temperature in the dark. Figure 2 gives the equilibrium adsorption time curves which were obtained by following the decrease of the concentration of bilirubin within the plasma samples with time. As seen here, there were relatively faster adsorption rates observed at the beginning, and then adsorption equilibria were achieved gradually in about 1 hr. Notice that there was a very low nonspecific bilirubin adsorption (i.e., the adsorption onto the unmodified polyamide hollow-fiber membranes), which was about 0.65 mg bilirubin per gram polymer (Curve A, Fig. 2). There are no reactive functional groups onto the unmodified polyamide hollow-fiber membranes, which interact with bilirubin molecules, hence this adsorption may be due to diffusion of bilirubin into the pores and weak interactions between hydrophobic bilirubin molecules and amide groups on the surface of hollow fibers. On the other hand, much higher adsorption capacities were obtained when the Cibacron Blue F3GA-attached polyamide hollow-fibers were used (Curves B and C, Fig. 2).

Figure 3 gives the bilirubin adsorption capacities of the adsorbent polyamide hollow-fiber membranes carrying different amounts of Cibacron Blue F3GA. Note that the adsorption capacities were evaluated using the initial and equilibrium concentrations of bilirubin in the adsorption media. As seen here, when the number of Cibacron Blue F3GA molecules on the polyamide hollow-fiber membranes increased, the amount of adsorbed bilirubin also increased in the studied region, as expected and then reached almost a constant value. This may

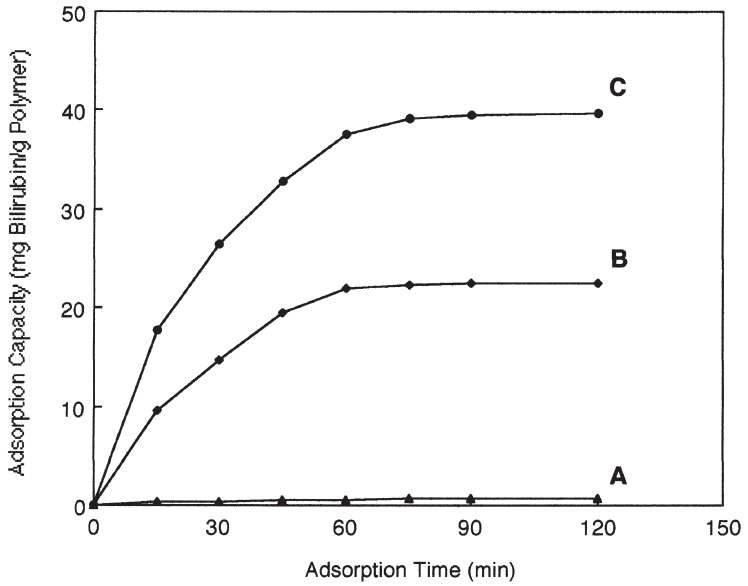


Figure 2. Equilibrium adsorption times of bilirubin from human plasma: bilirubin initial concentration: 17.8 mg 100 mL^{-1}; temperature: 25°C; curve A: adsorption onto unmodified polyamide hollow fibers; curve B: adsorption onto polyamide with 23.5 μmol Cibacron Blue F3GA per gram polymer; curve C: adsorption onto polyamide with 42.5 μmol Cibacron Blue F3GA per gram polymer.

be explained as follows; when the dye loading increases, the attached amount of hydrophobic groups on the membrane surface, which will interact with the bilirubin will increase leading to higher bilirubin adsorption. Maximum bilirubin adsorption capacity was 39.7 mg g^{-1}.

Effects of Bilirubin Initial Concentration on Adsorption

Figure 4 shows the nonspecific and specific adsorption of bilirubin onto polyamide hollow-fiber membranes. The amount of bilirubin adsorption on the unmodified polyamide hollow-fiber membranes was about 0.65 mg g^{-1} polymer, while much higher adsorption values of up to 39.7 mg bilirubin g^{-1} were achieved in the case of the Cibacron Blue F3GA-attached hollow-fiber membranes. The specific bilirubin adsorption increased with the bilirubin initial concentration and reached a plateau (at 8.9 mg bilirubin 100 mL^{-1}), at which we may assume that all the active points available for bilirubin

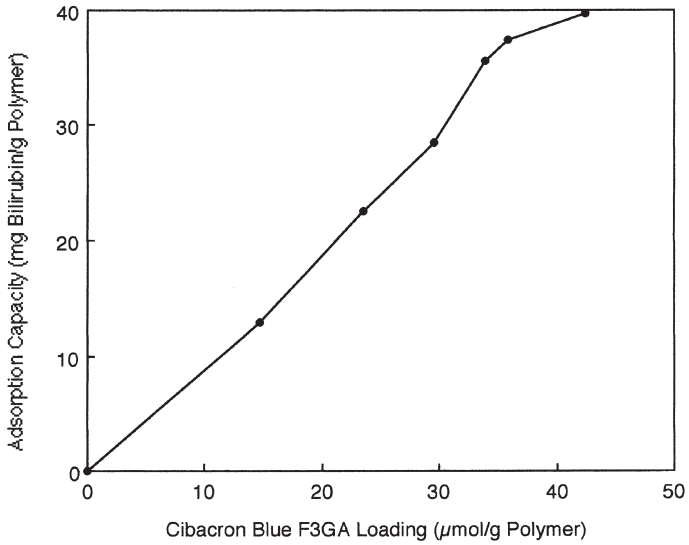


Figure 3. Effect of Cibacron Blue F3GA loading on bilirubin adsorption: bilirubin initial concentration: 17.8 mg 100 mL^{-1}; temperature: 25°C.

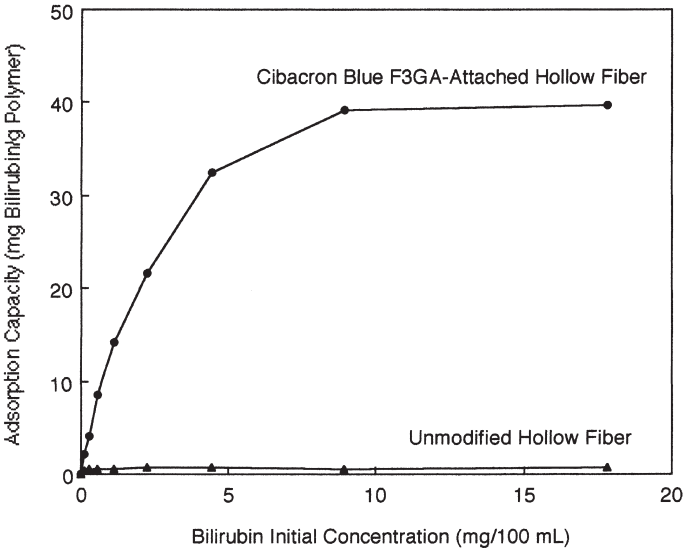


Figure 4. Effect of bilirubin initial concentration on adsorption: Cibacron Blue F3GA loading: 42.5 μmol per gram polymer; temperature: 25°C.

adsorption were occupied by bilirubin molecules. The high bilirubin concentration may also contribute to this high bilirubin adsorption due to the high driving force between the human plasma and solid phases (i.e., polyamide hollow-fiber membrane).

Note that a wide variety of sorbents with a wide range of adsorption capacities were reported in literature for bilirubin removal. Davies et al. presented adsorption capacities of 4.0–80 mg bilirubin g^{-1} with their anion-exchange resins (31). Chandy and Sharma reached adsorption capacities of 0.66–1.13 mg bilirubin g^{-1} with the polylysine-immobilized chitosan beads (14). Zhu et al. have reported 0.2–75 mg bilirubin g^{-1} with the polypeptide (i.e., poly-L-lysine, poly-D-lysine and poly-L-ornithine) coated polyamide resin (3). Henning et al. have showed 5–80 mg bilirubin g^{-1} with the polyamide resins containing various basic amino acids (32). Sideman et al. reported bilirubin adsorption capacities between 2 and 24 mg g^{-1} with a macroreticular resin (12). Kanai et al. have developed an improved model of anion-exchange resin (IONEX) and they obtained the maximum amount of bilirubin adsorption as 7.7 mg g^{-1} (33). Kuroda et al. have synthesized macroporous glycidyl methacrylate-divinylbenzene copolymer beads for bilirubin removal and they showed 30 mg g^{-1} bilirubin adsorption capacity (19). Denizli et al. used poly(hydroxyethylmethacrylate) based dye affinity microbeads and they obtained 6.8–32.5 mg g^{-1} polymer for bilirubin removal from human plasma (34–37). The maximum bilirubin adsorption that we achieved with the sorbent system developed in this study was 48.9 mg bilirubin g^{-1} polymer which was quite comparable with the related literature.

Bilirubin vs. Albumin Adsorption

It is generally accepted that bilirubin exists in the serum in two forms: direct and indirect. The direct reacting type is thought to be bilirubin conjugated with glucuronic acid, rendering it water soluble, while the indirect is bound to blood protein, albumin (13,14). It is reported that some sorbents like activated carbon can remove bilirubin only from the free or soluble phase, and the removal efficiency is limited by the tight binding of bilirubin to albumin (38). The ideas of removing of bilirubin by using oligopeptide pentands as ligand in preparation of affinity sorbents (13), or alternatively adsorption of albumin–bilirubin conjugates have also been utilized (4). Starting from the same point, we selected Cibacron Blue F3GA as the affinity ligand, which was shown as a good ligand for affinity separation of albumin in our previous studies (39,40). In addition, we were expecting a further increase in the bilirubin removal by direct interaction of bilirubin molecules with the attached Cibacron Blue F3GA molecules.

In order to observe the interrelation between albumin and bilirubin adsorptions, we also followed the changes of albumin concentration in the plasma samples before and after each adsorption cycle. Human serum albumin adsorption was $224\,mg\,g^{-1}$ polymer. The total protein adsorption was determined as $236\,mg\,g^{-1}$. Almost in all cases, the ratio of the numbers (as μmol) of bilirubin molecules to albumin molecules adsorbed on the sorbent hollow fibers were in the range of 40–50. Note that according to the related literature, each albumin molecule may have as many as 12 binding sites for bilirubin, but only two of the sites bind bilirubin molecules tightly (41). This is very significantly higher in our case, which means that, there may be adsorption of albumin–bilirubin conjugates, but, bilirubin molecules are preferentially adsorbed by our ligand, i.e., Cibacron Blue F3GA, in direct interaction. Note that there is an equilibrium between the free and albumin-conjugated bilirubin, therefore when one removes the free form by using sorbents, more bilirubin molecules will be released from the albumin-conjugates in order to attain this equilibrium, which, we believe, was also the case in our system. This process will continuously strip bilirubin molecules from the protein conjugate until the adsorption equilibrium between the free bilirubin, the albumin-conjugated bilirubin, and the sorbent is reached.

Effect of Temperature on Bilirubin Adsorption

The effect of temperature on bilirubin adsorption was also studied. In these experiments, we used the plasma with a total initial bilirubin concentration of $17.8\,mg\,100\,mL^{-1}$. The Cibacron Blue F3GA-attached polyamide hollow-fiber membranes containing 42.5 μmol Cibacron Blue F3GA/g were incubated with this plasma. The bilirubin adsorption curves obtained at three different temperatures, i.e., 4, 25, and 37°C are shown in Fig. 5. The amount of adsorbed bilirubin per unit amount of the sorbent increases with increasing temperature. Note that the maximum bilirubin adsorption was 48.9 mg bilirubin per gram polymer at 37°C. In general, adsorption decreases as temperature increases (42), but in bilirubin case, it was different. Takase and Baba have found increased bilirubin adsorption with increasing temperature (43). Davies et al. examined the effects of temperature on bilirubin removal from solution by an anion exchange (31). They also showed that bilirubin adsorption increased with temperature. One hypothesis is that a conformational change takes place in the bilirubin molecule (44). The bilirubin molecule changed from a *cis* configuration to a *trans* configuration with increasing temperature. This would allow for lessened streric hindrance in the binding of bilirubin to the attached Cibacron Blue F3GA molecules.

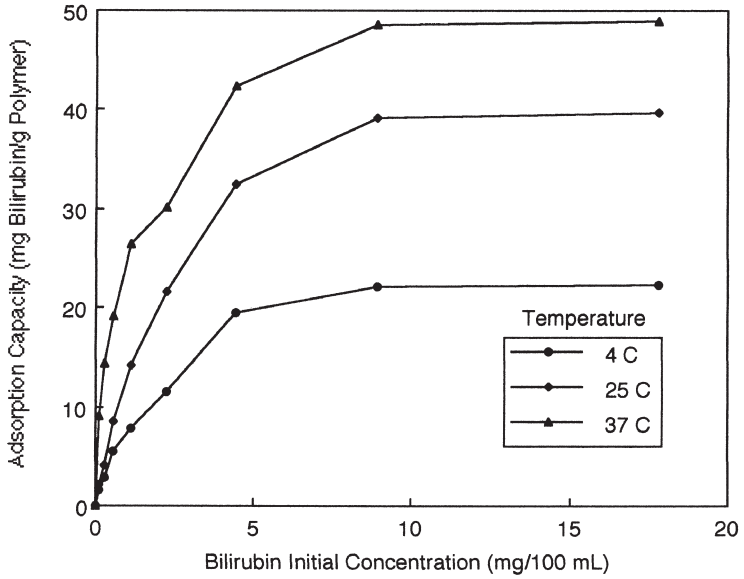


Figure 5. Effect of temperature on bilirubin adsorption: Cibacron Blue F3GA loading: 42.5 μmol per gram polymer; bilirubin initial concentration: 17.8 mg 100 mL^{-1}.

CONCLUSION

Hollow-fiber membranes with well-defined pore structures and specifically functionalized surfaces play an important role in hemoperfusion. In this study, we prepared a new adsorbent system, which composed of Cibacron Blue F3GA as the specific dye-ligand and polyamide hollow-fibers as the support matrix. The results presented in this communication showed that upto 48.9 mg bilirubin per unit mass of the adsorbent can be adsorbed at relatively low equilibrium adsorption times. It was possible to adsorb more bilirubin at higher temperatures. The preliminary batch-wise experiments allowed us to conclude that this inexpensive adsorbent system may be an important alternative to the existing bioaffinity adsorbents in the therapy of hyperbilirubinemia. Further studies using hollow-fiber modules in extracorporeal recirculation units are under investigation.

REFERENCES

1. Ostrow, J.D. *Bile Pigments: Jaundice, Molecular Metabolic and Medical Aspects*; Marcel Dekker: New York, 1986.

2. Berk, P.D. A Computer Simulation Study Relating to the Treatment of Fulminant Hepatic Failure by Hemoperfusion. Proc. Soc. Exp. Biol. Med. **1977**, *155*, 535–540.
3. Zhu, X.X.; Brown, G.R.; St-Pierre, L.E. Adsorption of Bilirubin with Polypeptide Coated Resins. Biomater. Artif. Cells Artif. Organs **1990**, *18*, 75–93.
4. Klinkman, H.; Falkenhagen, D.; Courtney, J.M. Blood Purification by Hemoperfusion. Int. J. Artif. Organs **1979**, *2*, 296–308.
5. Xu, C.X.; Tang, X.J.; Niu, Z.; Li, Z.M. Studies of Adsorbents for Haemoperfusion in Artificial Liver Support I. Preparation and In-Vitro Studies of Cross-Linked Agarose Beads Entrapped Activated Charcoal. Int. J. Artif. Organs **1981**, *4*, 200–204.
6. Yueming, C.; Xianjue, T.; Changxi, X.; Zhong Ming, L. Preparation and Performance of Cross-Linked Agar Encapsulated Activated Charcoal. J. Microencapsul. **1991**, *8*, 327–334.
7. Sideman, S.; Mor, L.; Mihich, M.; Lupovich, S.; Brandes, J.M.; Zeltzer, M. Resin Hemoperfusion for Unconjugated Bilirubin Removal. Contrib. Nephrol. **1982**, *29*, 90–100.
8. Sideman, S.; Mor, L.; Brandes, J.M. Removal of Bilirubin by Hemoperfusion with Ion Exchange Resins. Trans. Am. Soc. Artif. Intern. Organs **1979**, *25*, 497–500.
9. Clas, S.D.; Henning, D.S.; Brown, G.R.; St-Pierre, L.E. Polymer Resins with Aminoacid Containing Pendants for Sorption of Bilirubin IV. Site Binding Constants. Biomater. Artif. Cells Artif. Organs **1989**, *17*, 137–151.
10. Henning, D.S.; Clas, S.D.; Brown, G.R.; St-Pierre, L.E. Polymer Resins with Aminoacid Containing Pendants for Sorption of Bilirubin III. Adsorption and Desorption in the Presence of Albumin. Biomater. Artif. Cells Artif. Organs **1987**, *15*, 677–686.
11. Idezuki, Y.; Hamaguchi, M.; Hamabe, S.; Moriya, H.; Nagashima, T.; Watanabe, H.; Sonoda, T.; Teramoto, K.; Kikuchi, T.; Tanzawa, H. Removal of Bilirubin and Bile Acid with a New Anion Exchange Resin: Experimental Background and Clinical Experiences. Trans. Am. Soc. Artif. Intern. Organs **1981**, *27*, 428–433.
12. Sideman, S.; Mor, L.; Mordohovich, D.; Mihich, M.; Zinder, O.; Brandes, J.M. In Vivo Hemoperfusion Studies of Unconjugated Bilirubin Removal by Ion Exchange Resin. ASAIO **1981**, *27*, 434–438.
13. Brown, G.R. Oligo-peptide Functionalized Polymeric Sorbents for Bilirubin—A Review. Int. J. Biochromatogr. **1994**, *1*, 73–81.
14. Chandy, T.; Sharma, C.P. Polylysine Immobilized Chitosan Beads as Adsorbents for Bilirubin. Artif. Organs **1992**, *16*, 568–576.
15. Yamazaki, Z.; Inoue, N.; Wada, T.; Oda, T.; Atsumi, K.; Kataoka, K.; Fujisaki, Y. Use of an AR-1 Resin Column to Reduce Bilirubin-Level in Modified Ascitic Fluid. Trans. Am. Soc. Artif. Intern. Organs **1979**, *25*, 480–486.

16. Morimoto, T.; Matsushima, M.; Sowa, N.; Ide, K.; Sawanishi, S. Plasma Adsorption Using Bilirubin-Adsorbent Materials as a Treatment for Patients with Hepatic Failure. Artif. Organs **1989**, *13*, 447–452.
17. Mor, L.; Thaler, I.; Brandes, J.M.; Sideman, S. In Vitro Hemoperfusion Studies for Bilirubin Removal from Jaundiced Dogs. Int. J. Artif. Organs **1981**, *4*, 125–128.
18. Yu, Y.; He, B.; Gu, H. Adsorption of Bilirubin by Amine Containing Chitosan Resins. Artif. Cells Blood Substit. Immobil. Biotechnol. **2000**, *28*, 307–320.
19. Kuroda, H.; Ranaka, T.; Osawa, Z. Selective Adsorption of Bilirubin by Macroporous Poly(glycidyl methacrylate-co-divinyl benzene) Beads. Angew. Makromol. Chem. **1996**, *237*, 143.
20. Zeng, X.; Ruckenstein, E. Supported Dye-Affinity Membranes and Their Protein Adsorption Properties. J. Membr. Sci. **1996**, *117*, 271–286.
21. Kassab, A.; Yavuz, H.; Odabasi, M.; Denizli, A. Human Serum Albumin Chromatography by Cibacron Blue F3GA-Derived Microporous Polyamide Hollow Fiber Affinity Membranes. J. Chromatogr. B **2000**, *746*, 123–132.
22. Kugel, K.; Moseley, A.; Harding, G.B.; Klein, E. Microporous Poly(caprolactom) Hollow Fibers for Therapeutic Affinity Adsorption. J. Membr. Sci. **1992**, *74*, 115–122.
23. Marios, M., (Ed.) *Affinity Chromatography*; Elsevier: Amsterdam, The Netherlands, 1993.
24. Petsch, D.; Beeskow, T.C.; Anspach, F.B.; Deckwer, W.D. Membrane Adsorbers for Selective Removal of Bacterial Endotoxin. J. Chromatogr. B **1997**, *693*, 79–87.
25. Marios, M., (Ed.) *Dye-Ligand Chromatography*; Amicon Corporation: Lexington, 1980.
26. Lowe, C.R.; Small, D.A.P.; Atkinson, A. Some Preparative and Analytical Applications of Triazine Dyes. Int. J. Biochem. **1981**, *13*, 33–40.
27. Denizli, A.; Pişkin, E. Dye–Ligand Affinity Systems. J. Biochem. Biophys. Methods **2001**, *49*, 391–416.
28. LeDain, M.Y.; Kindbeiter, J.M.; Heerspink, W.; Schweizer, E.; Eizenwiener, H.G.; Ann. Biol. Clin. **1985**, *43*, 618.
29. Tietz, N.W. *Textbook of Clinical Chemistry*; WB Saunders Comp.: Philadelphia, 1986; 589–596.
30. Denizli, A.; Köktürk, G.; Yavuz, H.; Pişkin, E. Dye Ligand Column Chromatography: Albumin Adsorption from Aqueous Media and Human Plasma with Poly(EGDMA-HEMA) Microbeads. J. Appl. Polym. Sci. **1999**, *74*, 2803–2810.
31. Davies, C.R.; Malchesky, P.S.; Saide, G.M. Temperature and Albumin Effects on Adsorption of Bilirubin from Standard Solution Using Anion-Exchange Membrane. Artif. Organs **1990**, *14*, 14–19.

32. Henning, D.S.; Brown, G.R.; St-Pierre, L.E. Polymer Resins with Amino Acid Containing Pendants for Sorption of Bilirubin II. Polyamide Resins with Various Basic Aminoacids. Int. J. Artif. Organs **1986**, *9*, 33–38.
33. Kanai, F.; Takahama, T.; Iizuka, I.; Hiraishi, M.; Yamazaki, Z.; Fujimori, Y.; Maruyama, Y.; Wada, T.; Asano, K.; Sonoda, T.; Artif. Organs **1985**, *9*, 75.
34. Kocakulak, M.; Denizli, A.; Rad, A.Y.; Pişkin, E. New Sorbent for Bilirubin Removal from Human Plasma: Cibacron Blue F3GA-Immobilized Poly(EGDMA–HEMA) Microbeads. J. Chromatogr. B **1997**, *693*, 271–276.
35. Denizli, A.; Kocakulak, M.; Pişkin, E. Alkali Blue 6B-Derivatized Poly(EGDMA–HEMA) Microbeads for Bilirubin Removal from Human Plasma. J. Macromol. Sci., Pure Appl. Chem. **1998**, *A35*, 137–149.
36. Denizli, A.; Kocakulak, M.; Pişkin, E. Specific Sorbents for Bilirubin Removal from Human Plasma: Congo Red Modified Poly(EGDMA–HEMA) Microbeads. J. Appl. Polym. Sci. **1998**, *68*, 373–380.
37. Denizli, A.; Kocakulak, M.; Pişkin, E. Bilirubin Removal from Human Plasma in a Packed Bed Column System with Dye-Affinity Microbeads. J. Chromatogr. B **1998**, *707*, 25–31.
38. Miles, D.R.; Dorson, W.J.; Brandon, T.A.; Druyor, R.L.; Pizziconi, V.B. An Efficient Method for Removing Bilirubin. ASAIO Trans. **1990**, *6*, M611–M615.
39. Denizli, A.; Köktürk, G.; Yavuz, H.; Pişkin, E. Albumin Adsorption from Aqueous Solutions and Human Plasma in a Packed-Bed Column with Cibacron Blue F3GA-Zn(II) Attached Poly(EGDMA–HEMA) Microbeads. React. Functl. Polym. **1999**, *40*, 195–203.
40. Tuncel, A.; Denizli, A.; Purvis, D.; Lowe, C.R.; Pişkin, E. Cibacron Blue-F3GA Attached Monosize Polyvinylalcohol-Coated Polystyrene Microspheres for Specific Albumin Adsorption. J. Chromatogr. **1993**, *634*, 161–168.
41. Broderson, R. Bilirubin Solubility and Interaction with Albumin and Phospholipid. J. Biol. Chem. **1979**, *254*, 2364–2369.
42. Adamson, A.W. *Physical Chemistry of Surfaces*; 4th Ed.; John Wiley and Sons: New York, 1982.
43. Takase, S.; Baba, S. A Study of Bilirubin Adsorption in Bilirubin Adsorbents BR-601. In *Proceedings of the 5th Symposium on Plasmapheresis, Tokyo*; Oda, T., Ed.; 1985; 175–178.
44. Willson, R.A.; Hofmann, A.F.; Kuster, G.G.R. Toward an Artificial Liver II. Removal of Cholephilic Anions from Dogs with Abstruction by Hemoperfusion Through Charged and Uncharged Resins. Gastroenterology **1974**, *66*, 95–101.

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