Specific Sorbents for Bilirubin Removal from Human Plasma: Congo Red-Modified Poly(EGDMA/HEMA) Microbeads

ADIL DENIZLI,[1] MUSTAFA KOCAKULAK,[2] ERHAN PIŞKIN[2]

[1] Department of Chemistry, Biochemistry Division, Hacettepe University, Beytepe, Ankara, Turkey

[2] Chemical Engineering Department and Bioengineering Division, Hacettepe University, Beytepe, Ankara, Turkey

Received 14 March 1997; accepted 5 October 1997

ABSTRACT: Congo Red-modified poly(EGDMA–HEMA) microbeads were investigated as a specific sorbent for bilirubin removal from human plasma. Poly(EGDMA–HEMA) microbeads were prepared by a modified suspension copolymerization technique. Congo Red was covalently incorporated into the poly(EGDMA–HEMA) microbeads via condensation reactions between the aromatic amine groups of the dyes and the hydroxyl groups of the HEMA, under alkaline conditions. Bilirubin adsorption was investigated from hyperbilirubinemic human plasma on the poly(EGDMA–HEMA) microbeads containing different amounts of attached Congo Red (between 2.5 and 14.6 μmol/g). The nonspecific bilirubin adsorption on the unmodified poly(EGDMA–HEMA) microbeads were 0.32 mg/g from human plasma. High adsorption rates were observed at the beginning, and the adsorption equilibrium was then gradually achieved in about 30–60 min. Much higher bilirubin adsorption values, up to 11.7 mg/g, were obtained with the Congo Red-modified microbeads especially at 37°C. The numbers (as μmol) of bilirubin molecules to albumin molecules adsorbed on the sorbent microbeads were in the range of 15–20, which showed that bilirubin molecules were preferentially adsorbed to the Congo Red-modified microbeads. Bilirubin adsorption increased with increasing temperature. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 373–380, 1998

Key words: hyperbilirubinemia; bilirubin removal; specific sorbents; Congo Red-modified poly(EGDMA–HEMA) microbeads

INTRODUCTION

A partially functioning liver is usually unable to clear the body of bilirubin, a product of the catabolic breakdown of hemoglobin. Large concentrations of bilirubin in the blood have, therefore, been associated with hepatic failure and with neurological brain damage of newborn babies suffering from hemolysing diseases.[1] Bilirubin is a yellow–orange bile pigment. The free bilirubin is toxic. It is transported to the liver as a complex with albumin where it is normally conjugated and excreted into the bile.[2] There have been many attempts to remove bilirubin directly from the plasma of patients suffering from hyperbilirubinemia by hemoperfusion treatment, that is, the circulation of blood through an extracorporeal unit containing an adsorbent system for bilirubin.[3–17] Albumin-immobilized agarose,[4] activated charcoal,[5] and agar[6] have been used as sorbents in hemoperfusion columns. In most cases, basic ion-exchange resins were utilized.[7,8] Uncharged resins have been shown to adsorb bilirubin from aqueous media.[9,10] Idezuki et al. used anion-exchange syn-

Correspondence to: A. Denizli, P.K. 51, Samanpazari, 06242, Ankara, Turkey.

Journal of Applied Polymer Science, Vol. 68, 373–380, (1998)
 CCC 0021-8995/98/030373-08

thetic fibers and clinically applied this sorbent system in a selective bilirubin separation.[11] Sideman et al. suggested the application of hemoperfusion for the removal of bilirubin from jaundiced newborn babies using albumin-deposited macroreticular resin.[12] Brown prepared oligopeptide-functionalized polyacrylamide beads as an affinity sorbent system for bilirubin removal.[13] Chandy and Sharma used polylysine-immobilized chitosan beads for selective bilirubin removal.[14] Yamazaki et al. developed poly(styrene–divinylbenzene)-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–divinylbenzene 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] Plotz et al. conjugated human serum albumin with agarose using cyanogen bromide and reported a high bilirubin binding capacity.[17]

In this study, we developed an alternative sorbent for bilirubin removal. We selected Congo Red as the affinity ligand, which was shown to be a good ligand for affinity separation of albumin in our previous studies.[18–22] In addition, we were expecting a further increase in bilirubin removal by the direct interaction of bilirubin molecules with immobilized Congo Red molecules. Poly(EGDMA–HEMA) microbeads were selected as the carrier matrix, which were produced by suspension polymerization as also described in our earlier publications.[22–25] In the present article, we report our preliminary experiments related to the bilirubin-adsorption behavior of this new sorbent, in which plasma samples were obtained from a patient with hyperbilirubinemia.

EXPERIMENTAL

Preparation of Congo Red-Modified Microbeads

Poly(EGDMA–HEMA) microbeads were selected as the carrier matrix for the synthesis of an affinity sorbent for bilirubin removal. The microbeads were produced by a modified suspension polymerization of the respective comonomers, that is, ethylene glycol dimethacrylate (EGDMA, Rohm, Germany) and 2-hydroxyethyl methacrylate (HEMA, Sigma, St. Louis, MO) in an aqueous media as described in our previous articles.[22–25] Benzoyl peroxide (BPO) and poly(vinyl alcohol) (PVAL) (M_n: 100.000, 98% hydrolyzed, Aldrich, Rockford, IL) were used as the initiator and the stabilizer, respectively. Toluene (Merck AG, Darmstadt, Germany) was utilized as the diluent and used as received. The dispersion medium was distilled water. To produce polymeric microbeads of about 150–200 μm in diameter and with a narrow size distribution, the amounts of EGDMA, HEMA, toluene, water, BPO, and PVAL were 8 mL, 4 mL, 12 mL, 50 mL, 0.06 g, and 0.2 g, respectively. Polymerizations were carried out at an agitation rate of 600 rpm at 65°C for 4 h and at 90°C for 2 h. After cooling, the polymeric microbeads were separated from the polymerization medium by filtration, and the residuals (e.g., unconverted monomer and toluene) were removed by a cleaning procedure given in detail elsewhere.[26]

Congo Red was used as the specific affinity ligand which was obtained from BDH (UK). Three grams of poly(EGDMA–HEMA) microbeads was magnetically stirred (at 400 rpm) in a sealed reactor at a constant temperature of 80°C for 4 h with 100 mL of the Congo Red aqueous solution containing 4.0 g NaOH. To change the extent of Congo Red immobilization, the initial concentration of the Congo Red in the medium was varied between 0.1 and 4.0 mg/mL. After incubation, the Congo Red-modified microbeads were filtered and washed with distilled water and methanol several times until all the physically attached Congo Red molecules were removed. The dye-modified microbeads were stored at 4°C with 0.02% sodium azide to prevent microbial contamination. The leakage of the Congo Red from the microbeads was followed by treating the microbeads with fresh human plasma samples for 24 h at room temperature. Congo Red leakage after this treatment was measured in the liquid phase spectrophotometrically at 630 nm. The amount of Congo Red on the microbeads was evaluated using an elemental analysis instrument (Leco, CHNS-932, USA) by considering the nitrogen and sulfur stoichiometries.

Bilirubin Removal from Human Plasma

Bilirubin removal from human plasma with the unmodified and Congo Red-modified poly(EGDMA–HEMA) microbeads was studied batchwise. The blood samples were obtained from patients with hyperbilirubinemia. The plasma was separated by centrifugation at 500 g for 30 min at room temperature. Since bilirubin is destroyed

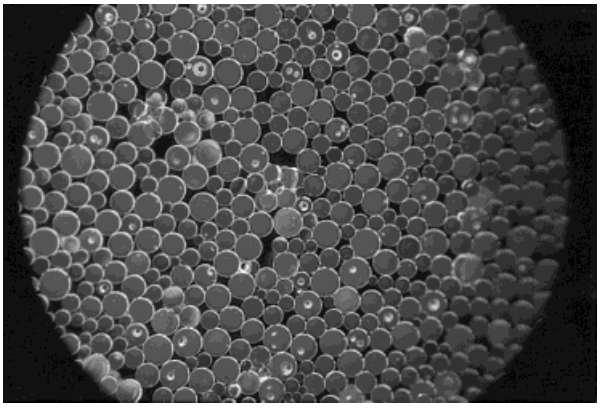

Figure 1 A representative optical micrograph of poly-(EGDMA–HEMA) microbeads.

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 plasma freshly separated from the patient were incubated with a 100 mg of the unmodified and Congo Red-modified poly(EGDMA–HEMA) microbeads at different temperatures (i.e., 4, 25, and 37°C) for 2 h. Poly-(EGDMA–HEMA) microbeads containing different amounts of Congo Red on their surfaces were utilized. The amounts of bilirubin removed were determined by a Malloy/Evelyn modified colorimetric test by measuring the decrease in the bilirubin concentration in the plasma samples.[27] Total protein and albumin concentrations in the plasma samples both before use and after treatment were determined by Biuret and brom cresol green methods, respectively.[28,29]

RESULTS AND DISCUSSION

In this study, we attempted to prepare a specific sorbent for bilirubin removal from patients with hyperbilirubinemia. Congo Red was used as the affinity ligand for the specific binding of bilirubin molecules. Poly(EGDMA–HEMA) microbeads were selected as the carrier matrix. Details of preparation and characterization of both the unmodified and Congo Red-modified poly(EGDMA–HEMA) microbeads were given in our previous articles.[18–22] Figure 1 shows a representative optical micrograph of these novel sorbent microbeads.

Chemical structures of Congo Red and bilirubin molecules are shown in Figure 2. Congo Red molecules were covalently incorporated into the poly-(EGDMA–HEMA) microbeads. Covalent bonds were formed as a result of the condensation reactions between the aromatic amine groups of the dyes and the hydroxyl groups of the HEMA, under alkaline conditions.[18–21] Bilirubin is a hydrophobic molecule due to intramolecular hydrogen bonds. The bilirubin molecule has a nonpolar backbone with two carboxylic groups which are partly ionized in the physiological blood pH.[30] Hence, the adsorption of bilirubin on the Congo Red-incorporated poly(EGDMA–HEMA) sorbent can either be based on physical attachment due to London Forces between the nonpolar bilirubin molecules and the hydrophobic sites on the Congo Red or by electrical attraction between the carboxyl groups on the bilirubin molecules and the amino groups on the Congo Red molecules.

Bilirubin Adsorption

Adsorption Rate

In this group of experiments, we used human plasma samples obtained from a patient with hyperbilirubinemia, in which the total bilirubin concentration was 19.5 mg/100 mL. Unmodified and Congo Red-modified poly(EGDMA–HEMA) microbeads were incubated with the plasma samples for 2 h at room temperature in the dark. Figure 3 gives the adsorption rate curves which were obtained by following the decrease of the concentration of bilirubin within the plasma samples with

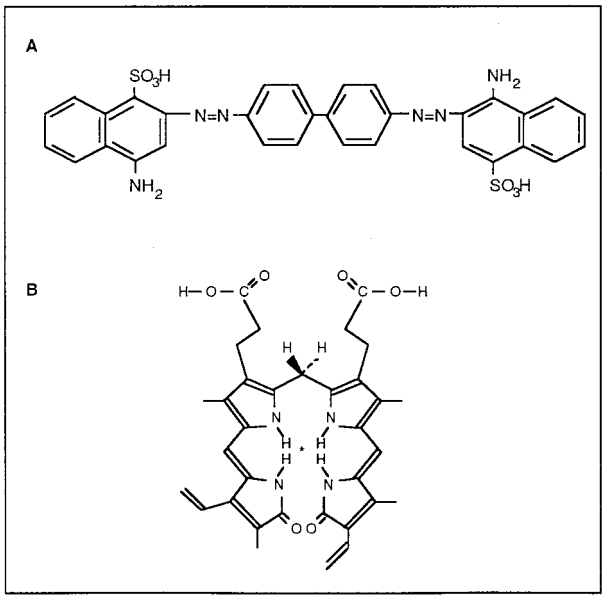

Figure 2 Chemical structures of (A) Congo Red and (B) bilirubin.

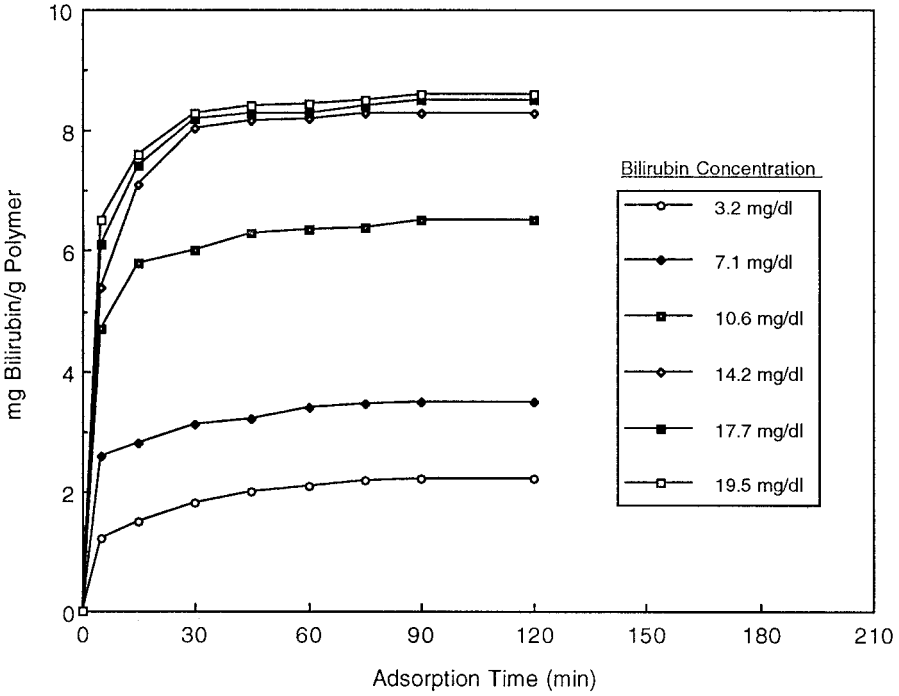

Figure 3 Adsorption rates of bilirubin from human plasma containing different amounts of bilirubin. Ligand surface concentration: 14.5 μmol Congo Red/g polymer; temperature: 25°C.

time. These curves indicate once again that the adsorption process was completed within about 1 h and this value can be considered as the equilibrium time for bilirubin adsorption. It can be seen that the adsorption rate increased with increasing bilirubin concentration, which may be due to a high driving force, which is the bilirubin concentration difference between the liquid (i.e., the plasma) and the solid (i.e., the microbeads) phases, in the case of high bilirubin concentration.

Adsorption Capacity

Effect of Ligand Surface Concentration. Figure 4 gives the adsorption capacities of the sorbent microbeads carrying different amounts of Congo Red. 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 Congo Red molecules on the microbeads increased, the amount of adsorbed bilirubin also increased in the studied region, as expected, due to the number of available active functional groups on the Congo Red ligands for interaction with bilirubin molecules.

Effects of Bilirubin Initial Concentration. In this group of studies, the unmodified poly(EGDMA–HEMA) and Congo Red-modified microbeads were incubated with the human plasma samples containing different amounts of bilirubin (1.6–19.5 mg/100 mL). Bilirubin solutions were obtained by dilution of the plasma.

Figure 5 shows the bilirubin-adsorption isotherms for unmodified and Congo Red-modified poly(EGDMA–HEMA) microbeads. Note that the albumin adsorption capacities of Congo Red-modified microbeads is also given as an enlarged graph in the figure. Notice that there was a very low nonspecific bilirubin adsorption [i.e., the adsorption onto the unmodified poly(EGDMA–HEMA) microbeads] which was about 0.32 mg bilirubin/g polymer. There is no functional groups on the unmodified poly(EGDMA–HEMA) microbeads which interact with the bilirubin molecules; hence, this adsorption may be due to the diffusion of bilirubin into the swollen matrix and weak interactions between bilirubin and hydroxyl groups on the surface of the microbeads. On the other hand, much higher adsorption values up to 8.6 mg bilirubin/g were achieved in the case of the Congo Red-modified microbeads. The specific bilirubin adsorption increased with the bilirubin initial concentration and reached a plateau (at around 15 mg bilirubin/100 mL), at which we may assumed that all the active points available for

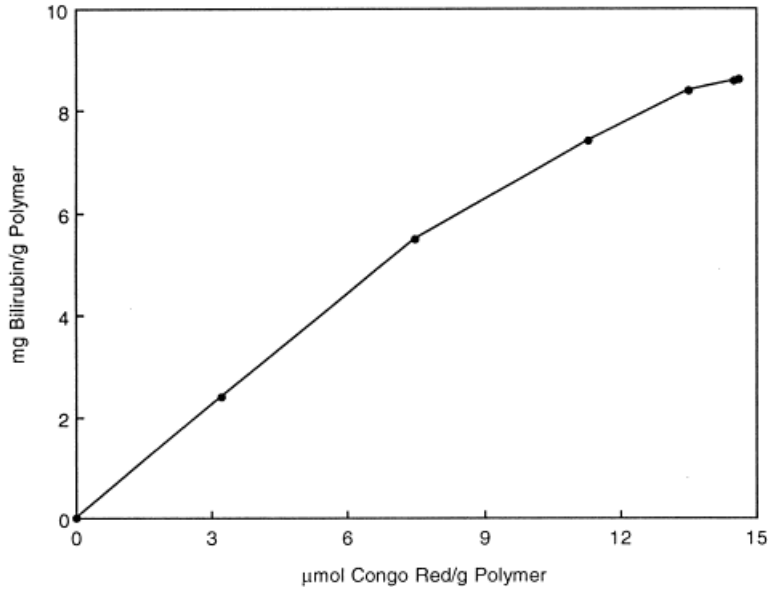

Figure 4 Effect of Congo Red surface concentration on bilirubin adsorption. Bilirubin initial concentration: 19.5 mg/100 mL; temperature: 25°C.

bilirubin adsorption were occupied with bilirubin molecules.

Note that a wide variety of sorbents with a wide range of adsorption capacities were reported in the literature for bilirubin removal. Dunlop reported a bilirubin-adsorption capacity between 0.3 and 5 mg/g with charcoal.[31] The hydrophobic and ionic properties of bilirubin makes it natural to try uncharged resins and anion-exchangers as a bilirubin sorbent. Davies et al. presented the adsorption capacities of 4.0–80 mg bilirubin/g with their anion-exchange resins.[32] Chandy and

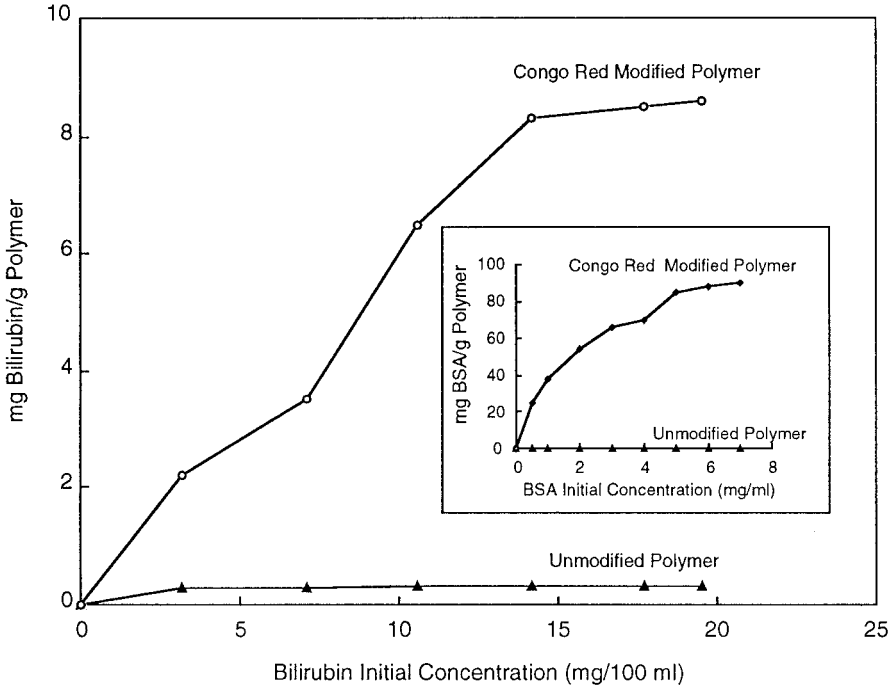

Figure 5 Effect of bilirubin initial concentration on adsorption. Ligand surface concentration: 14.5 μmol Congo Red/g polymer; temperature: 25°C.

Sharma reached adsorption capacities of 0.66–1.13 mg bilirubin/g with polylysine-immobilized chitosan beads.[14] Zhu et al. reported 0.2–75 mg bilirubin/g with polypeptide (i.e., poly-L-lysine, poly-D-lysine, and poly-L-ornithine)-coated polyamide resin.[3] Henning et al. showed 5–80 mg bilirubin/g with polyamide resins containing various basic amino acids.[33] Sideman et al. reported bilirubin-adsorption capacities between 2 and 24 mg/g with a macroreticular resin.[12] Kanai et al. developed an improved model of an anion-exchange resin and they obtained a maximum amount of bilirubin of 7.7 mg/g.[34] Hughes et al. showed a 0.3-mg bilirubin-adsorption capacity per g albuminated agarose from plasma.[35] The maximum bilirubin adsorption that we achieved with the sorbent system developed in this study was 8.6–11.7 mg bilirubin/g polymer, which was quite comparable with the related literature.

Bilirubin Versus Albumin Adsorption. Bilirubin exists in the serum in two forms: direct and indirect. The direct 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] 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.[36] Ideas of removing bilirubin by using oligopeptide pentands as a ligand in the preparation of affinity sorbents[13] or, alternatively, the adsorption of albumin–bilirubin conjugates have also been reported.[4] Starting from the same point, we selected Congo Red as the affinity ligand, which was shown to be a good ligand for the affinity separation of albumin in our previous studies.[18–21] In addition, we were expecting a further increase in bilirubin removal by the direct interaction of bilirubin molecules with the immobilized Congo Red molecules.

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. The albumin-adsorption capacity of the Congo Red-modified microbeads was in the range of 25–90 mg BSA/g polymer (Fig. 5). The total protein adsorption was parallel to the albumin adsorption. Almost in all cases, the ratio of the numbers (as μmol) of bilirubin molecules to albumin molecules adsorbed on the sorbent microbeads was in the range of 15–20. Note that according to the related literature each albumin molecule can bind two bilirubin molecules.[37] This is 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, that is, Congo Red, in a 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 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 among the free bilirubin, the albumin-conjugated bilirubin, and the sorbent is reached.

Effects of Temperature on Bilirubin Adsorption. The effects of temperature on bilirubin adsorption was also studied. In these experiments, we used the plasma with a total initial bilirubin concentration of 19.5 mg/100 mL. Congo Red-modified poly (EGDMA–HEMA) microbeads were incubated with this plasma. The bilirubin-adsorption curves at 4, 25, and 37°C, representing the relative amount of bilirubin adsorbed with respect to the bilirubin initial concentrations, are shown in Figure 6. The amount of adsorbed bilirubin per unit amount of the sorbent increases with increasing temperature. Note that the maximum bilirubin adsorption was 11.7 mg bilirubin/g polymer.

In general, adsorption decreases as temperature increases,[38] but in the bilirubin case, it was different. Takase and Baba found increased bilirubin adsorption with increasing temperature.[39] Davies et al. examined the effects of temperature on bilirubin removal from a solution by anion exchange.[32] They also showed increased adsorption with temperature. As the temperature is increased, the adsorption curve changes to the single species adsorption curve characteristic of Langmuir adsorption. This reflects a change in the mechanism of adsorption. One hypothesis is that a conformational change takes place in the bilirubin molecule.[40] The bilirubin molecule went from a *cis* to a *trans* configuration with increasing temperature. This would allow for lessened streric hindrance in the binding of bilirubin to the Congo Red molecules.

CONCLUSION

In this study, we developed a new sorbent system which was composed of Congo Red as the specific

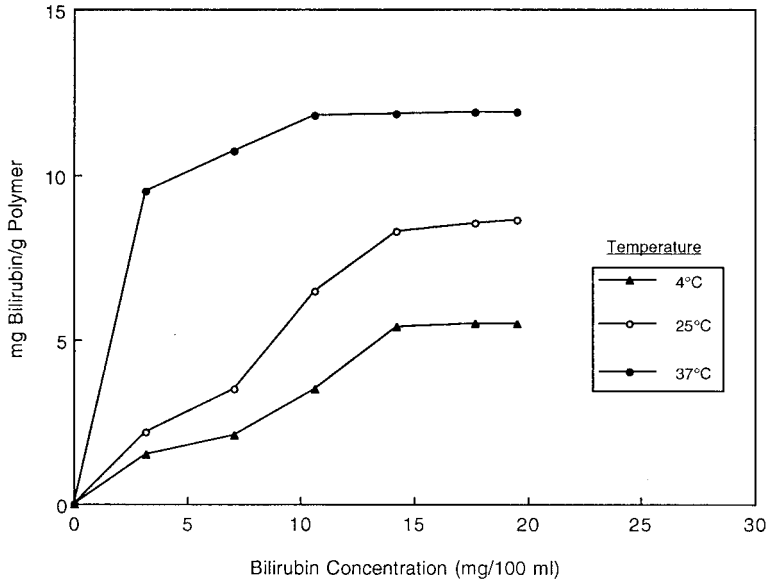

Figure 6 Effect of temperature on bilirubin adsorption. Ligand surface concentration: 14.5 μmol Congo Red/g polymer; bilirubin initial concentration: 19.5 mg/100 mL.

ligand and poly(EGDMA–HEMA) microbeads as the carrier matrix. The results presented in this communication showed that up to 11.7 mg bilirubin per unit mass of the sorbent can be adsorbed at relatively high adsorption rates. The numbers (as μmol) of bilirubin molecules to albumin molecules adsorbed on the sorbent microbeads were in the range of 15–20, which means that there may be adsorption of albumin–bilirubin conjugates to the sorbent microbeads, but bilirubin molecules were preferentially adsorbed by our ligand, that is, Congo Red, in a direct interaction. It was possible to adsorb more bilirubin at higher temperatures. The preliminary batchwise experiments allowed us to conclude that this inexpensive sorbent system may be an important alternative to the existing sorbents in the therapy of hyperbilirubinemia. Further studies using packed-bed columns filled with the dye-modified sorbents in extracorporeal recirculation units are under investigation.

REFERENCES

1. J. D. Ostrow, *Bile Pigments and Jaundice Metabolic and Medical Aspects,* Marcel Dekker, New York, 1986.
2. P. D. Berk, *Proc. Soc. Exp. Biol. Med.,* **155,** 535 (1977).
3. X. X. Zhu, G. R. Brown, and L. E. St-Pierre, *Biomat. Artif. Cells Artif. Organs,* **18,** 75 (1990).
4. D. Bihari, R. D. Hugher, A. E. S. Gimson, P. G. Langley, R. J. Ede, G. Eder, and R. Williams, *Int. J. Artif. Organs,* **6,** 299 (1983).
5. C. X. Xu, X. J. Tang, Z. Niu, and Z. M. Li, *Int. J. Artif. Organs,* **4,** 200 (1981).
6. C. Yueming, T. Xianjue, X. Changxi, and L. Zhong Ming, *J. Microencap.,* **8,** 327 (1991).
7. S. Sideman, L. Mor, M. Minich, D. Mordohovich, S. Lupovich, J. M. Brandes, and M. Zeltzer, *Contr. Nephrol.,* **29,** 90 (1982).
8. S. Sideman, L. Mor, and J. M. Brandes, *Trans. Am. Soc. Artif. Intern. Organs,* **25,** 497 (1979).
9. S. D. Clas, D. S. Henning, G. R. Brown, and L. E. St-Pierre, *Biomater. Artif. Cells Artif. Organs,* **17,** 137 (1989).
10. D. S. Henning, S. D. Clas, G. R. Brown, and L. E. St-Pierre, *Biomater. Artif. Cells Artif. Organs,* **15,** 677 (1987).
11. Y. Idezuki, M. Hamaguchi, S. Hamabe, H. Moriya, T. Nagashima, H. Watanabe, T. Sonoda, K. Teramoto, T. Kikuchi, and H. Tanzawa, *Trans. Am. Soc. Artif. Intern. Organs,* **27,** 428 (1981).
12. S. Sideman, L. Mor, D. Mordohovich, M. Mihich, O. Zinder, and J. M. Brandes, *Trans. Am. Soc. Artif. Intern. Organs,* **27,** 434 (1981).
13. G. R. Brown, *Int. J. Biochromatog.,* **1,** 73 (1994).
14. T. Chandy and C. P. Sharma, *Artif. Organs,* **16,** 568 (1992).
15. Z. Yamazaki, N. Inoue, T. Wada, T. Oda, K. Atsumi, K. Kataoka, and Y. Fujisaki, *Trans. Am. Soc. Artif. Intern. Organs,* **25,** 480 (1979).
16. T. Morimoto, M. Matsushima, N. Sowa, K. Ide, and S. Sawanishi, *Artif. Organs,* **13,** 447 (1989).
17. P. H. Plotz, P. Beck, B. F. Scharschmidt, J. K. Gor-

don, and J. Vergalla, *J. Clin. Invest.,* **53,** 786 (1974).
18. A. Denizli, G. Köktürk, B. Salih, A. Kozluca, and E. Pişkin, *J. Appl. Polym. Sci.,* **63,** 27 (1997).
19. A. Denizli, B. Salih, and E. Pişkin, *J. Appl. Polym. Sci.,* **65,** 2085 (1997).
20. A. Denizli, B. Salih, and E. Pişkin, *J. Biomater. Sci., Polym. Ed.,* **8,** 411 (1997).
21. A. Denizli, A. Tuncel, A. Kozluca, K. Ecevit, and E. Pişkin, *Sep. Sci. Tech.,* **32,** 1003 (1997).
22. A. Denizli, B. Salih, and E. Pişkin, *J. Chromatogr.,* **731,** 57 (1996).
23. A. Denizli, B. Salih, and E. Pişkin, *React. Funct. Polym.,* **29,** 11 (1996).
24. B. Salih, A. Denizli, B. Engin, and E. Pişkin, *React. Funct. Polym.,* **27,** 199 (1995).
25. B. Salih, A. Denizli, and E. Pişkin, *Sep. Sci. Tech.,* **31,** 715 (1996).
26. A. Denizli, A. Y. Rad, and E. Pişkin, *J. Chromatogr. B,* **668,** 13 (1995).
27. M. Y. LeDain, J. M. Kindbeiter, W. Heerspink, E. Schweizer, and H. G. Eizenwiener, *Ann. Biol. Clin.,* **43,** 618 (1985).
28. B. Josephson and C. Gyllensward, *Scand. J. Clin. Lab. Invest.,* **9,** 29 (1957).
29. B. T. Dumas, W. A. Watson, and H. G. Biggs, *Clin. Chim. Acta,* **31,** 87 (1971).
30. S. Sideman, L. Mor, and J. M. Brandes, in *The Past, Present and Future of Artificial Organs,* E. Pişkin and T. M. S. Chang, Eds., Meteksan, Ankara, 1983.
31. E. H. Dunlop, *Biomed. Eng.,* **10,** 213 (1975).
32. C. R. Davies, P. S. Malchesky, and G. M. Saide, *Artif. Organs,* **14,** 14 (1990).
33. D. S. Henning, G. R. Brown, and L. E. St-Pierre, *Int. J. Artif. Organs,* **9,** 33 (1986).
34. F. Kanai, T. Takahama, I. Iizuka, M. Hiraishi, Z. Yamazaki, Y. Fujimori, Y. Maruyama, T. Wada, K. Asano, and T. Sonoda, *Artif. Organs,* **9,** 75 (1985).
35. R. D. Hughes, H. Y. Ton, and R. Williams, *Proc. Eur. Soc. Artif. Organs,* **6,** 297 (1979).
36. D. R. Miles, W. J. Dorson, T. A. Brandon, R. L. Druyor, D. L. Page, and V. B. Pizziconi, *ASAIO Trans.,* **36,** M611 (1990).
37. M. Branca, A. Gamba, P. Manitto, D. Monti, and G. Speranza, *Biochim. Biophys. Acta,* **742,** 341 (1983).
38. A. W. Adamson, *Physical Chemistry of Surfaces,* 4th ed., Wiley, New York, 1982, pp. 373–377.
39. S. Takase and S. Baba, in *Proceedings of the 5th Symposium on Plasmapheresis,* Tokyo, T., Oda, Ed., 1985, pp. 175–178.
40. R. A. Willson, A. F. Hofmann, and G. G. R. Kuster, *Gastroenterology,* **66,** 95 (1974).