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Polylysine-immobilized Affinity Nylon Membrane used for Bilirubin Adsorption

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INTRODUCTION

Bilirubin, a yellow-orange bile pigment, 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]. It deposits in tissue, especially in the brain, and is toxic. Disorders in the metabolism of bilirubin, especially common among newborn infants, may cause jaundice, a yellow discoloration of the skin and other tissues.

Many attempts have been made to remove the bilirubin directly from plasma of patients suffering from hyperbilirubinemia, such as phototherapy, hemodialysis and hemoperfusion. Phototherapy renders the normally occurring bilirubin into water-soluble forms, referred to as photobilirubin, which can be excreted more easily [2], but it is generally applicable for mild cases of hyperbilirubinemia. Hyperbilirubinemia can also be treated by hemoperfusion, i.e. circulation of blood through an extracorporeal unit containing an adsorbent system for bilirubin [3]. Zhu et al. prepared the poly-L-lysine (PLL) coated resins, and investigated the adsorption behavior of bilirubin. Their results indicated that the PLL coated resins have an improved binding affinity for bilirubin [4].

In recent years, microporous membranes are modified and various affinity ligands are coupled for use as alternative adsorbents for biomedical applications [5]. In contrast with conventional hemoperfusion columns, affinity membrane separation has a unique and powerful role as support tool in the removal of toxic substances from human plasma, due to elimination internal diffusion limitations.

In this study, nylon microfiltration membranes are activated with epibromohydrin at terminal amino groups. Hydroxyethyl celluloses (HEC) are covalently immobilized on the activated membranes to yield HEC-coated membrane matrices. Then, immobilization of PLL onto HEC-coated membranes via epibromohydrin activation provides PLL affinity membrane. Such PLL–HEC affinity membranes are set in stack and used to adsorb bilirubin from the phosphate buffer solutions. The purpose of this work is to study adsorption behavior on the nylon affinity membranes.

EXPERIMENTAL

Materials

Nylon membranes (47 mm diameter, 0.45 μm pore size) were obtained from Whatman (England).
Hydroxyethyl cellulose was provided by Fluka (Switzerland). Poly-L-lysine and sodium cyanoborohydride were purchased from Sigma (Germany). Bovine serum albumin (BSA) was obtained from Beijing Chemical Reagent Company (China). Bilirubin was purchased from Shanghai Weihui Chemical Factory (China). The other reagents used were bought in China.

Methods

Preparation of HEC-bound Membrane

Nylon membrane disks (47 mm diameter) were shaken in 1 M HCl for 24 h at room temperature. After partial hydrolysis of amide bonds, the membranes were shaken in 20% (v/v) epibromohydrin solution, pH 11, adjusted by NaOH, at 333 K for 10 h. After activation, the membranes were washed three times for 20 min with water at room temperature.

The activated membranes were shaken in 2% (w/w) HEC solution, pH 11, for 30 min at room temperature. The HEC solution was then sucked through the membranes, which were subsequently incubated in an oven at 373 K for 10 h. Non-covalently bound HEC was removed by washing the membranes with 0.1 M NaOH and deionized water. The amount of HEC bound on the membranes was determined by the phenol-sulfuric acid method [6].

Immobilization of PLL

The HEC-bound membranes were activated with epibromohydrin again according to a procedure described by Beeskow et al. [7]. PLL was immobilized on the activated HEC-coated nylon membranes by the method of Pestch et al. [8]. The amount of PLL immobilized on the HEC-coated membrane was assayed by the ninhydrin method.

The reaction scheme for the preparation of affinity membrane is shown in Fig. 1.

RESULTS AND DISCUSSION

Effect of Temperature

The bilirubin adsorption curves obtained at 25 and 37°C are shown in Fig. 2. The amount of adsorbed bilirubin per unit amount of the sorbent increases with increasing temperature. In general, adsorption decreases as temperature increases, but in the case of bilirubin it was different. One hypothesis is that a conformational change takes place in the bilirubin molecule configuration with increasing temperature. This would allow for lessened steric hindrance in the binding of bilirubin to the attached PLL molecules.

Effect of Ionic Strength

The effect of the ionic strength on bilirubin adsorption is presented in Fig. 3, which shows that the adsorption capacity decreases with increasing NaCl concentration in bilirubin solution. The binding of bilirubin to PLL is primarily achieved by electrostatic interactions between the positively charged functional groups of the constituent amino acids and the negatively charged carboxyl groups on the bilirubin molecule. When the NaCl concentration changes from 0.05 to 0.4 M, the adsorption of bilirubin decreases by 12%. The decrease in the adsorption capacity as the ionic strength increases can be attributed to the weaker electrostatic interaction between the PLL-attached membranes and bilirubin molecules.

FIGURE 1 Reaction scheme for the preparation of affinity membrane. Matrix-NH₂ is nylon membrane; R−OH is HEC and H₂N−Y is PLL.

FIGURE 2 Effect of temperature on the bilirubin adsorption capacities on PLL-attached membrane. Phosphate buffer: 0.066 mol/l (pH 7.4); bilirubin solution: 20 ml 100 mg/l; membrane: 0.10 g.
Analysis of the Adsorption Mechanism

Freundlich adsorption isotherms are applied for the description of the adsorption mechanism for bilirubin on PLL-attached membrane. The isotherms can be described as follows:

\[
\frac{q}{m} = Kc^{\frac{1}{n}}
\]  (1)

where \( q \) is adsorption capacity (mg) of bilirubin; \( m \) is the weight of the membrane in grams; \( c \) is the bilirubin concentration; \( n \) and \( K \) are the physical constants of Freundlich adsorption isotherm.

Equation (1) can be transformed into Eq. (2)

\[
\log \frac{q}{m} = \log K + \frac{1}{n} \log c
\]  (2)

Figure 4 shows the linear relationship of the Freundlich isotherm for the adsorption of bilirubin with PLL-attached membranes. This indicates that the adsorption mechanism is a monolayer adsorption. According to Fig. 4, one obtains \( K = 4.152 \), and \( n = 2.3262 \).

Effect of Feed Rate on Adsorption

Bilirubin-phosphate buffer solution (200 mg/l) was loaded onto the affinity stack of 10 membranes with 47 mm diameter in a single-pass mode at constant flow-rate. The effect of flow rate on the adsorption of bilirubin was studied and the breakthrough curves are shown in Fig. 5. From the figure, we notice that the breakthrough curves became sharper and equilibrium was reached faster with increase in the rate of feed, but the amount of bilirubin adsorbed decreased. A reasonable explanation is that local equilibration can be achieved and ligands on the membrane surface and in pores can be utilized efficiently at low flow rate, while at high flow rate, the retarding time was so short that bilirubin had not enough time to make contact with the ligands and utilization ration of the ligands decreased.

CONCLUSIONS

Activated membranes for covalent immobilization of HEC were obtained by reaction of microfiltration nylon membranes with epibromohydrin. Polylsine was immobilized onto the HEC-coated membrane by epibromohydrin activation. The affinity membranes were used to adsorb the bilirubin from the phosphate buffer solutions. The batch experiments results showed that the mechanism of adsorption of bilirubin with PLL-attached membranes is a monolayer adsorption. Breakthrough curves revealed that these membranes can well remove the bilirubin from the phosphate buffer solutions, and high feed rate...
was not suitable for affinity membrane chromatography.

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References


