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New Sorbent for Bilirubin Removal from Human Plasma: Albumin Immobilized Microporous Membranous PTFE Capillaries

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Abstract: In this study, we developed a tailored capillary sorbent for bilirubin removal. For immobilized bioligand, capillaries were grafted with epoxy groups using RIGP. The HSA immobilized capillaries has a high affinity adsorption capacity (71.2 mg bilirubin/g polymer) and a shorter adsorption equilibrium time (about 60 min).

Keywords: Bilirubin removal, radiation grafting, PTFE capillaries, human serum albumin.

Bilirubin (BR), a bile pigment, is formed as a result of the catabolism of hemoglobin[1]. High free BR concentration may cause organic dysfunction and brain damage[2]. In recent years, (bio) affinity chromatography technique has developed into a powerful tool for the removal of toxins directly from human plasma[3]. Various affinity-ligands were coupled to different bead or membrane matrixes. However, they have a number of drawbacks. For example, bead matrixes usually require high pressure equipments, complex packed procedure, and membranes also need complex membrane cartridge.

In order to overcome these problems, the present work aims at preparation of new adsorbent for BR removal which composed of human serum albumin (HSA) as the ligand and microporous membranous polytetrafluoroethylene capillaries (MPTFE) as the matrix. In order to attach the affinity-ligand, we chose a radiation-induced graft polymerization (RIGP) technique to introduce the reactive epoxy groups onto the surface of MPTFE. After this procedure, HSA was immobilized onto the capillaries. All affinity MPTFE was investigated for BR removal in a flow injection system.

Experimental

The capillaries (immersed in 40 wt% GMA/MeOH solution) were irradiated by γ-rays (Co-60 source, 60000 Ci, USTC, China). The radiation grafting was carried out in N_2 medium at room temperature. Dose rate was 5 kGy/h and the total dose was varied up to 100 kGy. The unreacted monomer and homopolymers were removed with THF in Soxhlet apparatus. The degree of grafting (d.g.) is defined as follows:

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$$\text{Degree of grafting (d.g.) (\%)} = [(W_g - W_0)/ W_0] \cdot 100 \quad (1)$$

W_g and W_0 denote the masses of the grafted and the ungrafted MPTFE, respectively. The capillaries were preconditioned with MeOH[4], then 5 mL incubation buffer (0.2 mg/mL HSA, 100 mmol/L phosphate, pH 7.0) was recirculated though the capillaries for 2 h. After this step, the capillaries were incubated with 5 mL fresh HSA solution for 18 h. Remove the non-covalently bound protein fraction with the washing solution (incubation buffer supplemented with 0.1% Tween-20 (w/w), 150 mmol/L NaCl). The Ponceau S method was applied to determine the amount of bound protein[5]. The adsorption studies were carried out in a flow injection system (Model FI-2100, Haiguang Instrument Co.), and it was equipped with a water jacket for controlling temperature. All the adsorption experiments were carried out in dark. In a typical experiment, 50 mL plasma (having different BR concentrations) was recirculated through the capillary for 2 h. The concentration of BR and albumin in the plasma were determined by using HITACHI 7060 automated analyzer.

Results and Discussion

MPTFE is a new special material, has both advantages of membrane and micro-column, as described in our previous papers[6]. Starting from this point, we selected MPTFE as the affinity matrix.

Figure 1 showed the dependence of the d.g. of GMA onto MPTFE for irradiation method. It can be seen that, the d.g. reaches a plateau value above which the increase of the dose has no effect. The binding capacities for HSA onto MPTFE at different d.g. are given in **Figure 2**. The adsorption capacity experiments were carried out at different flow-rates. With the increasing of the flow-rate, the adsorption capacity of sorbents decreased significantly. The reason may be that the decrease of residence time in the capillary makes the time of interaction between BR and the sorbent to be not long enough.

Figure 1 Dependence of the d.g. of GMA onto PTFE on irradiation dose

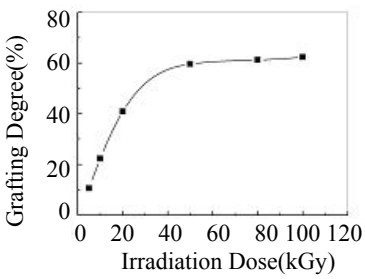
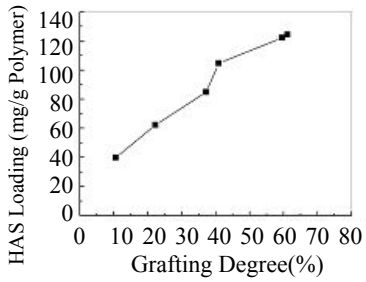


Figure 2 Dependence of the amount of HSA loading on the d.g.



The effect of temperature on the adsorption of BR experiment was carried out at different temperatures (*i.e.* 4℃, 25℃, 37℃). The result displayed that the adsorption capacity of sorbents increased with increasing temperature. We obtained the maximum

BR adsorption (84.4 mg BR/g polymer) at 37℃. Proverbially, the adsorption capacity decreases with increasing temperature, but in our case it was different. We proposed that a conformational change took place in the BR molecule[7]. The configuration of the BR molecule changed from *cis* to *trans* with increasing temperature. This would lessen steric hindrance for binding BR to HSA molecules. **Figure 3** showed the adsorption rate curves, which were obtained by following the changes of the concentration of BR within the plasma with time. As seen in **Figure 3**, adsorption equilibrium is achieved in about 60 min for MPTFE. **Figure 4** showed the non-specific and specific adsorption of BR onto the unmodified and HSA-immobilized MPTFE. The adsorption capacity of the unmodified MPTFE was quite low (about 0.52 mg BR /g polymer), while much higher adsorption values (up to 71.2 mg BR/g polymer) were achieved in the modified MPTFE. The maximum BR adsorption, that we achieved with the sorbent system developed in this study, was quite comparable with the related literature [8-12].

Figure 3 Adsorption rates of BR from human plasma with different BR concentration

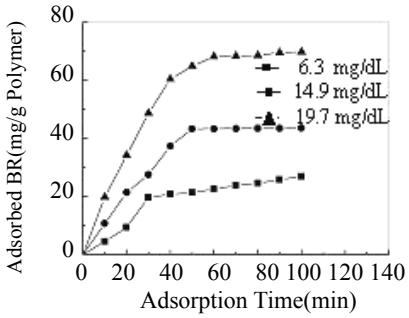
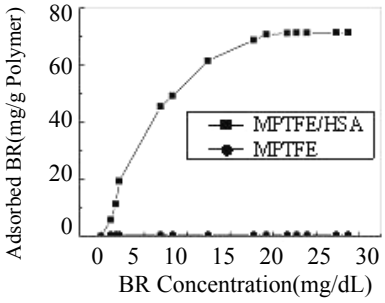


Figure 4 Effect of BR initial concentration on adsorption



In order to observe the interrelation between albumin and BR adsorptions, we also followed the changes of albumin concentration (200–500 mg/L) in the plasma before and after each adsorption cycle. Albumin adsorption was in the range of 3.3–3.6 mg HSA/g polymer, the BR adsorption was in the range of 53.2–64.3 mg BR/g polymer at the same time. The result displayed that the affinity capillary still maintains relatively higher adsorption capacity at high concentration solution of albumin.

Conclusion

HSA immobilized MPTFE capillaries have good affinity adsorption capacity for BR removal, and shorter adsorption equilibrium time. And this sorbent is inexpensive and easy to operate. Therefore, it has a potential merits in clinical application.

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