Chinese Chemical Letters Vol. 16, No. 8, pp 1085-1088, 2005
http://www.imm.ac.cn/journal/ccl.html

Preparation of Chitosan-coated Nylon Membranes and their Application as Affinity Membranes

Wei SHI[1,2], Feng Bao ZHANG[1]*, Guo Liang ZHANG[1]

[1]School of Chemical Engineering & Technology, Tianjin University, Tianjin 300072
[2]Biomedical Engineering Research Center, Medical College, Xiamen University, Xiamen 361005

Abstract: Chitosan-coated nylon membranes which possess a large number of reactive groups of $-CH_2OH$ and $-NH_2$ were prepared by coupling chitosan onto the nylon membrane. Then polylysine as ligand was also immobilized onto the composite membranes by 1, 1'-carbonyl-diimidazole activation to prepare affinity membranes for bilirubin adsorption. The results showed that these membranes exhibited high binding affinity capacities for bilirubin and the adsorption isotherm fitted the Freundlich model well.

Keywords: Chitosan, affinity membrane, bilirubin, adsorption.

As a new technology in affinity separation, the membrane affinity chromatography has proven its efficiency and time stability. This technology already has got significant applications in the separation and purification of biomolecules[1]. One of the most important factors in membrane affinity chromatography is the identification of suitable membranes. The selection of the membrane material and its preparation constitute dominant factors affecting the chromatographic performance[2]. Nylon membrane offers narrow pore size distribution and good mechanical rigidity. However, nylon membrane has a low concentration of primary amino groups leading to low ligand density[3]. Another disadvantage of this matrix is nonspecific adsorption of protein[4]. In order to solve these problems, nylon membranes are bound with polyhydroxyl-containing material, such as polyglucose, dextran, starch, cellulose and hydroxyethylcellulose[5] to increase reactive sites and reduce nonspecific adsorption. In this paper, nylon membrane was activated by epibromohydrin, then chitosan (CS) was coupled on the activated membrane to improve the hydrophilicity and increase reactive sites. Polylysine (PLL) as ligand was also immobilized onto the CS-coated nylon membrane. Such PLL-CS affinity membranes were used to adsorb bilirubin.

Materials

Nylon membranes (47 mm diameter, 0.45 μm pore size) were obtained from Whatman (England). Chitosan (Mr-400, 000) (CS) was provided by Fluka (Swizerland). Poly-

* E-mail: zhangfengbao@eyou.com

L-lysine (MW: 242000, DP: 1158) (PLL) and 1, 1'-carbonyldiimidazole (CDI) were purchased from Sigma (Germany). Bilirubin was purchased from Shanghai Weihui Chemical Factory (China). The other reagents used were bought in China.

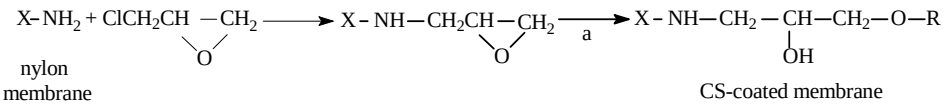
Preparation of Affinity-membrane

Nylon membrane disks were shaken in 1 mol/L HCl for 24 h at room temperature. After partial hydrolysis of amide bonds, the membranes were shaken in 20% epibromohydrin solution, pH 11, adjusted by NaOH, at 333K for 10 h. The activated membranes were shaken in 10 mL chitosan solution (prepared by dissolving 0.15 g CS in 10 mL 1 vol.% acetic acid solution) for 1 h at room temperature. The CS solution was then sucked through the membranes, which were subsequently incubated in an oven at 353K for 7 h. Non-covalently bound CS was removed by washing the membranes with 1 vol.% acetic acid solution and deionized water. The amount of CS bound on the membranes was determined by the ninhydrin method. The amount of CS coupled on the membrane was 92.6 mg/g nylon membrane. These membranes possess a large number of reactive groups of $–CH_2OH$ and $–NH_2$. Reaction scheme for the preparation of CS-coated nylon membrane is shown in **Scheme 1**.

The CS-coated membranes were shaken in a solution of 20 mg CDI per 10 mL acetone at room temperature for 1 h. After activation, the membranes were washed three times for 20 min with acetone at room temperature and then drying in vacuum.

An amount of 100 mg PLL was dissolved in 10 mL $NaHCO_3$ solution, pH 8.4. One CDI-activated CS-coated membrane was given in reaction solution and shaken overnight at room temperature. Afterwards the membranes were washed in 1 mol/L NaCl and water extensively. The amount of PLL immobilized on the CS-coated membrane was

Scheme 1 Reaction scheme for coupling of CS on nylon membrane

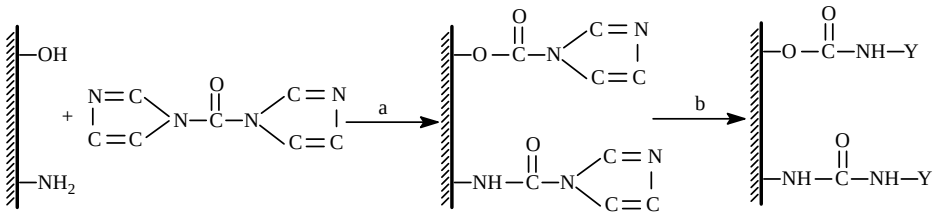


nylon membrane

CS-coated membrane

a) chitosan

Scheme 2 Activation of CS-coated membrane with CDI and covalent immobilization of PLL



CS-coated membrane

PLL-attached membrane

a) 20-25℃ b) PLL

assayed by the ninhydrin method. The content of PLL reached 89.1 mg/g nylon membrane. This result was great higher than that of HEC-coated nylon membranes[5,6]. Reaction scheme for the preparation of affinity membrane is shown in **Scheme 2**.

Result and Discussion

The PLL-attached membranes were tested for the adsorption of bilirubin in 0.066 mol/L phosphate buffer (pH 7.4) by batch experiment in a dark room.

Figure 1 showed the non-specific and specific adsorption of bilirubin onto the unmodified and PLL-attached membranes. The non-specific bilirubin adsorption on the membrane is quite low, and the amount was about 0.6 mg bilirubin/g unmodified membrane only. While much higher binding capacity, up to 32.4 mg bilirubin/g membrane was obtained after hydrophilic process in case of the PLL immobilization. The specific bilirubin adsorption increased with the increase of bilirubin initial concentration at the given concentration range.

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:

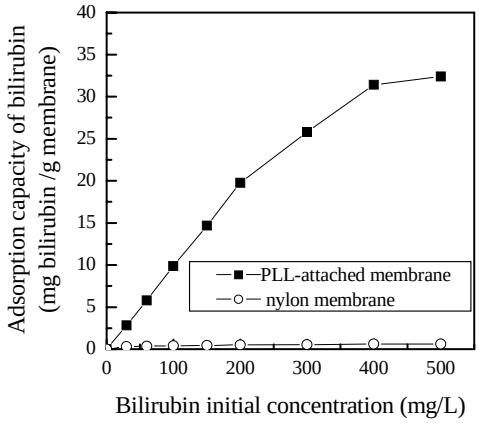
$$\frac{q}{m} = Kc^{\frac{1}{n}} \qquad (1)$$

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.

Eq 1 can be transformed into Eq 2

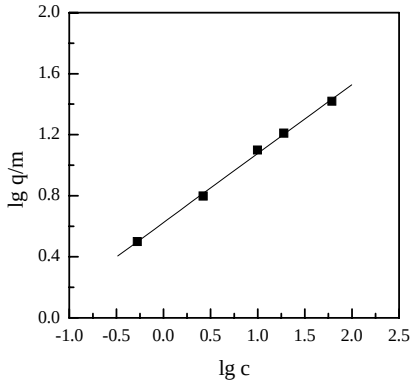
$$\lg\frac{q}{m} = \lg K + \frac{1}{n}\lg c \qquad (2)$$

Figure 1 The effect of bilirubin initial concentration on the adsorption capacities



Bilirubin solution volume: 5 mL, membrane weight: 0.050 g, temperature: 37℃.

Figure 2 Freundlich adsorption isotherm of PLL for bilirubin



Temperature: 37℃, bilirubin solution: 5 mL; membrane weight: 0.050 g.

Figure 2 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.

Acknowledgment

We are extremely grateful to the National Natural Science Foundation of China for supporting this research (No.29776036).

References

1. A. Tejeda, J. Ortega, I. Magana, R. Guzman, *J.Chromatogr. A*, **1999**, *830*(2), 293.
2. W. Guo, and E. Ruckenstein, *J. Membr. Sci.*, **2001**, *182* (1-2), 227.
3. H. Y. Gan, Z. H. Shang, J. D. Wang, *J. Chromatogr. A*, **2000**, *867*(1-2), 161.
4. T. C. Beeskow, W. Kusharyoto, F. B. Anspach, *et al.*, *J. Chromatogr. A*, **1995**, *715*(1), 49.
5. D. Petsch, T.C. Beeskow, F.B. Anspach, W. D. Deckwer, *J. Chromatogr. B*, **1995**, *693*(1), 79.
6. W. Shi, F. B. Zhang, G. L. Zhang, *et al.*, *Mol. Simulat.*, **2003**, *29*(12), 787.

Received 23 September, 2004