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Chemically Modified Chitosan Beads as Molecularly Imprinted Polymer Matrix for Adsorptive Separation of Proteins

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Abstract: In a phosphate buffer, a hemoglobin (Hb)-imprinted polymer complex was prepared using maleic anhydride (MAH) modified chitosan beads as matrix, acrylamide (AM) as functional monomer, N,N-methylenebisacrylamide (MBA) as cross-linker and potassiumpersulfate (KPS) / sodium hydrogen sulfite ($NaHSO_3$) as initiators. Langmuir analysis showed that an equal class of adsorption was formed in the molecular imprinting polymer (MIP), and the MIP has high adsorption capacity and selectivity for the imprinted molecule. The MIP can be reused and the recovery was approximately 100% at low concentration.

Keywords: Chemically modified chitosan beads, polyacrylamide, protein imprinting.

Molecular imprinting is an effective separation technique due to its specific molecular recognition[1,2]. In this paper we prepared a Hb-imprinted MIP, the MIP beads were characterized by FTIR, SEM. The adsorption capacity and the selectivity of the MIP were also discussed.

Experimental and Results

Chitosan beads were prepared and cross-linked as reported elsewhere [3,4], the chitosan beads were modified with maleic anhydride (MAH) to introduce vinyl groups. Chitosan beads, maleic anhydride and ethanol were put into a flask at 70℃, then added some amount of Et_3N, and heated to 80℃ for 8 h, the obtained MAH-chitosan beads were washed extensively with distilled water to remove remained reagents. For the preparation of Hb-imprinted polymer, some amount of modified chitosan beads, functional monomer Am, cross-linker MBA, oxidation agent KPS, imprinted molecule Hb were dissolved in 0.01 mol/L sodium dihydrogen phosphate buffer (pH 6.8). The mixture was stirred under a nitrogen atmosphere for 45 mins, then added 5 mL sodium dihydrogen phosphate buffer containing 0.16% (w/v) $NaHSO_3$ and stirred for 2 hours. The formed beads were put into a nylon stocking to press out the surrounding polyacrylamide gel, and the remained chitosan beads were washed with 10% (v/v) acetic acid containing 10% (w/v) sodium dodecyl sulfate (SDS) solution to desorb the Hb till

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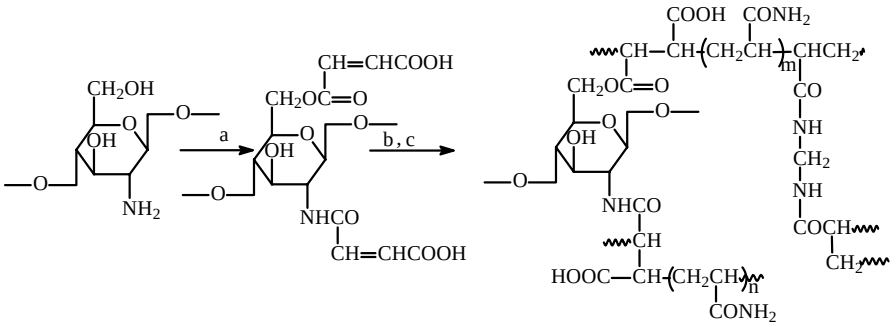
the color was pale. The non-imprinted polymer (P-O) was prepared using the recipe without addition of Hb. The modification process is depicted in **Scheme 1**.

To prove that acrylamide could be grafted on the MAH modified chitosan beads, only acrylamide, no cross-linker was added to react with the MAH modified chitosan, for the non-grafted polyacrylamide could be dissolved in water and washed out of the beads by elution. The FTIR spectra of the different chitosan beads are shown in **Figure 1**. The peak at 1569 cm^{-1} is brought by Vc=c, carbonyl groups and carboxylic groups were confirmed by the adsorption peaks at 1658 and 1704 cm^{-1} respectively. The strong peak of 1667 cm^{-1} shows the carbonyl groups of amides. The surface morphology of MIP beads is depicted in **Figure 2. Figure 2** showed that the surface of MIP beads is rough with pores.

The Langmuir adsorption curve of **Figure 3** shows that the Langmuir equation fits best for Hb adsorption on the MIP beads under the studied concentration range (correlation coefficient, r>0.99). The Q_{max} and b values can be calculated to be 35.8 mg/g wet beads and 1.44 mg/mL, respectively

Scheme 1



a) maleic anhydride b) acrylamide c) N,N-methylenebisacrylamide

Figure 1 FTIR spectra of (1) cross-linked chitosan beads, (2) MAH-chitosan beads and (3) polyacrylamide- grafted chitosan

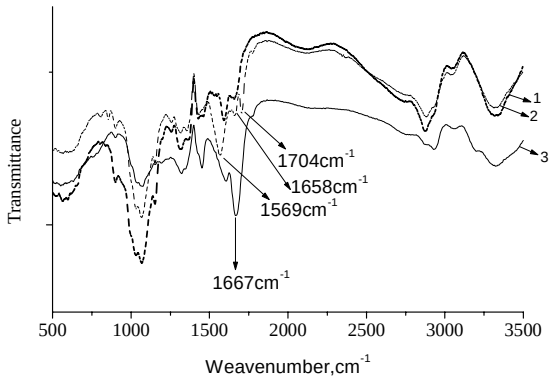


Figure 2 SEM of MIP beads

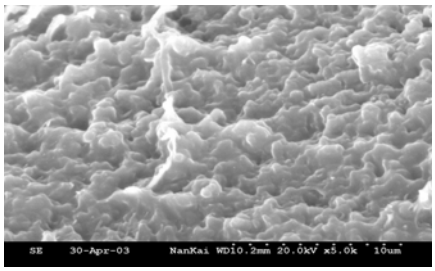
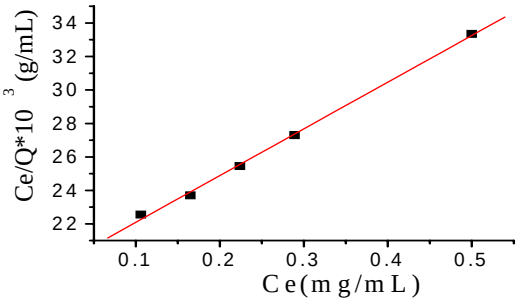


Figure 3 Langmuir adsorption curve of MIP



The special selectivity test of MIP was carried out using bovine serum albumin (BSA) as substrate. At the same condition (10 mL 1.0 mg/mL Hb, 0.5 g wet beads, 25°C for 16 hours), the adsorption capacity of the MIP for Hb and BSA was 5.4 mg and 0.7 mg, respectively compared to 0.34 mg and 0.023 mg of P-O. The data indicated that the imprinting methods create a microenvironment based on shape selection and position of functional groups which can recognize the imprinting molecule Hb. The MIP could be used many times and maintained their adsorption capacity at an almost constant value.

In conclusion, the Hb-imprinted polymer prepared in our lab exhibited high selective adsorption for Hb in a molecular recognition process and Hb could also be effectively adsorbed, such a MIP may be used to remove the protein from different solutions, it is also promising to use in the area of biosensor material.

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