Preparation of a Modified Chitosan and Polyvinyl Alcohol Affinity Membrane and Its Application in Purification of His-Tag Protein

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Abstract. In this work, a modified chitosan and polyvinyl alcohol membranes was prepared and employed for purification of His-Tag protein. First, the membranes were crosslinked with epichlorohydrin and covalently coupled with iminodiacetic acid. Then the Ni²⁺ was immobilized on membranes by chelation. The application of the affinity membrane for his-tag protein was studied. The effect of salt concentration and initial activity on His-Tag adsorption capacity was investigated. The results showed that the optimal loading conditions were 15°C, 0.6 mol/L NaCl, initial activity 1.0u/ml. The maximum adsorption is 35u/g.

Introduction

Recently, the affinity membrane has emerged as a new alternative technology to affinity column chromatography for simplifying the washing, elution and regeneration steps and improving the effectiveness [1].

Immobilized metal affinity chromatography (IMAC), which was based on specific adsorption between divalent metal ions and histidine, showed great potential for purifying His-tag protein. It has a number of advantages, such as low-cost, higher absorption capacity and easier scale-up. Chitosan is considered as one of the most prevalent membrane materials for its non-toxicity and high biocompatibility [2].

Serine hydroxymethyl transferase (SHMT) is an important biocatalyst which could transform glycine to serine at the presence of tetrahydrofolate, formaldehyde and pyridoxal phosphate. The His-tag was in its N-terminal and can be highly presented through E.coli BL21 (DE3).

Based on the issues mentioned above, a crosslinking chitosan and Polyvinyl alcohol chelated Ni²⁺ affinity membrane were prepared, Silica gel was used for Pore-agent. The adsorption capacity of Ni²⁺ and SHMT are investigated. Consequently, the modified chitosan transparent affinity membrane can be used for purifying the His-Tag protein.

Experimental

Preparation of the Modified Macroporous Chitosan and Polyvinyl Alcohol Membranes

The chitosan and polyvinyl alcohol membranes were prepared as follows. First dissolving 1.5g chitosan in 100mL 2% acetic acid solution; And 1g polyvinyl alcohol was dissolved in 30mL deionized water; Then, mixed the above two solutions together, until it to be homogeneous. Finally, the mixture was cultured in the 250ml flasks for 48 h at 50°C.

Afterwards, add 2.0g Silica gel to 40ml mixing solution, and cast it on a rimmed glass plate and dried at room temperature. The dried membrane was immersed in 2mol/L diluted sodium hydroxide solution to dissolve the silica gel at 50°C for 2h, and the membrane was washed out with deionized water (DI) . Lastly, the membrane was dried at room temperature and stored.
A piece of chitosan and polyvinyl alcohol membrane was incubated in a mixed solution of 5 ml epichlorohydrin and 20 ml 1 mol/L NaOH at 50°C for 2 h. After incubation, rinsed it with a lager of DI water.

For coupling IDA, the epichlorohydrin conjugated membrane was reacted with 25 ml of 0.2 M IDA and 1 M Na$_2$CO$_3$, pH 11, at 50°C for 12h. After reaction the membrane was washed with 5% acetic acid and then DI water$^4$.

**Results and Discussion**

![Figure 1. The chitosan and polyvinyl alcohol membranes.](image1)

![Figure 2. Section of chitosan and polyvinyl alcohol membranes.](image2)

Figure 1 shows the chitosan and polyvinyl alcohol membrane and scanning electron microscopy (JSM6510LV made in Japan) was employed to investigate the mophology. As a result, the pore structure could be maintained in Figure 2. (The membranes were fractured under liquid nitrogen)$^{[5,6]}$.

![Figure 3. XRD for unmodified membrane and modified membrane.](image3)

Figure 3 shows the XRD pattern (D8 Advance Germany brucker) transform of membrane before and after modification with epichlorohydrin. The reaction between chitosan and epichlorohydrin was observed with a new peak generating, it changed the crystal structure and increased the mechanical strength of affinity membrane$^{[7]}$. (The X-ray diffraction patterns were acquired at 45kV and 40mA with Cu Kα radiation λ=1.5406Å$^0$).
Figure 4 shows the effect of the initial concentration on Ni$^{2+}$ adsorption. The adsorption amounts on modified chitosan affinity membrane increased with increasing initial concentration and then stabilised in 0.1mol/L. The maximum adsorbed capability of Ni$^{2+}$ on modified affinity membrane is 60mg/g\(^{[8]}\).

Figure 5 shows the effect of the initial activity on SHMT adsorption. The adsorbed amounts on modified chitosan affinity membrane increased with initial activity and then levelled to stable constant values. The maximum adsorption occurred when the initial activity was 1.0u/ml, the adsorbed amount of 35u/g was achieved.

**Conclusions**

First Silica particles could be porogen of membrane. And epichlorohydrin changed the crystal shape of the chitosan and polyvinyl alcohol membrane. Second Ni$^{2+}$ as ligands was used to prepare IMAM for His-tag immobilized. The results showed that the maximum adsorption capacity of affinity membrane for SHMT was 35u/g in optimum absorption conditions at initial activity 1.0u/ml, 15°C, 0.6mol/L NaCl. All indications from the study show that modified chitosan and polyvinyl alcohol immobilized Ni$^{2+}$ affinity membrane are has the potential for purification His-tag.
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References


