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Facial synthesis of nickel(II)-immobilized carboxyl cotton chelator for purification of histidine-tagged proteins

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ABSTRACT

Immobilized metal affinity chromatography (IMAC) technique is frequently used in the purification of histidine-tagged (His-tagged) recombinant proteins. In this study, nickel(II)-immobilized carboxyl cotton chelator (CCC-Ni^{2+}) fibers was synthesized by a simple method based on the coordination effect between Ni^{2+} and carboxyl group. The nickel content of the CCC-Ni^{2+} fibers was determined to be 5 times larger than that of Ni^{2+}-immobilized sulfhydryl cotton fiber (SCF-Ni^{2+}) fibers developed in our previous work. The prepared CCC-Ni^{2+} fibers were then applied for the selective and rapid separation of His-tagged protein from escherichia coli (*E. coli*) cell lysates on the basis of the high affinity of Ni^{2+} to 6 × His with a lab-in-syringe format. Benefiting from the good biological compatibility and high nickel content, the results showed that CCC-Ni^{2+} fibers were able to selectively capture His-tagged proteins from complex *E. coli* cell lysates and exhibited a relatively large adsorption capacity toward His-tagged protein. The recoveries of His-tagged GFP in *E. coli* cell lysates were in the range of 89.8%-106.7% with the relative standard deviations (RSDs) less than 9.4% (intra-day) and 10.3% (inter-day). Taken together, this efficient approach for the purification of recombinant proteins extends the application of CCC-based fibrous materials in biological analysis.

1. Introduction

Immobilized metal affinity chromatography (IMAC), an important tool for protein purification, is based on the affinity of transition metal ions on the IMAC supports toward electron-donor groups on protein [1,2]. Thereinto, IMAC technique is frequently used in the purification of histidine-tagged (His-tagged) recombinant proteins, which is generally based on the affinity of Ni^{2+} ions toward a string of six histidine (6 × His) fused to the recombinant proteins [3–5]. His-tagged proteins can be selectively enriched by the IMAC adsorbent while untagged proteins are removed. Among these adsorbents, nitrilotriacetic acid (NTA)-attached resin with immobilized ions Ni^{2+}, is one of the most used adsorbent for separating His-tagged fusion proteins [5–8]. Although the NTA-Ni^{2+} resin can be used in various protein expression systems, it is still limited by a long separation time, solvent consumption and high cost [9,10].

To develop more rapid and convenient methods for the purpose, a variety of magnetic materials [11–15] have been exploited for the purification of His-tagged protein. However, the synthetic processes for most of the magnetic materials are complicated and time-consuming [16–18]. In addition, inorganic supports always suffer from the limitation of irreversible non-specific adsorption [1]. In this respect, natural fibrous material with excellent biological compatibility and good operability, can be an alternative matrix for biological research.

Cotton fiber is an important natural material and widely used as adsorbent due to its merits including high mechanical strength, stable chemical resistance and wide availability [19,20]. For example, a novel cotton fiber-packed pipet-tip was developed by Selmen's group and successfully applied for microscale enrichment of glycans and glycopeptides based on hydrophilic interaction [21]. However, the monotonous functional groups on the cotton fiber will restrict its broad applications. In this respect, sulfhydryl cotton fiber (SCF) was introduced as an IMAC support to immobilize Ni^{2+} ions based on the coordination effect between Ni^{2+} and thiol group, and the Ni^{2+}-immobilized sulfhydryl cotton fiber (SCF-Ni^{2+}) adsorbent was used for the selective enrichment of His-tagged proteins in our recent work [22]. Thanks to the high reactivity of thiol group, SCF was further functional by "thiol-ene" click chemistry to

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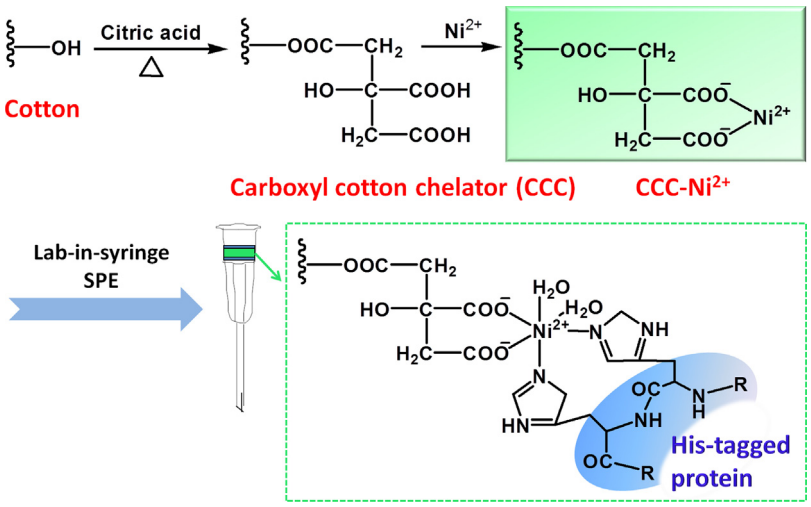

Fig. 1. Schematic diagram of the preparation of CCC-Ni^{2+} fibers and lab-in-syringe SPE.

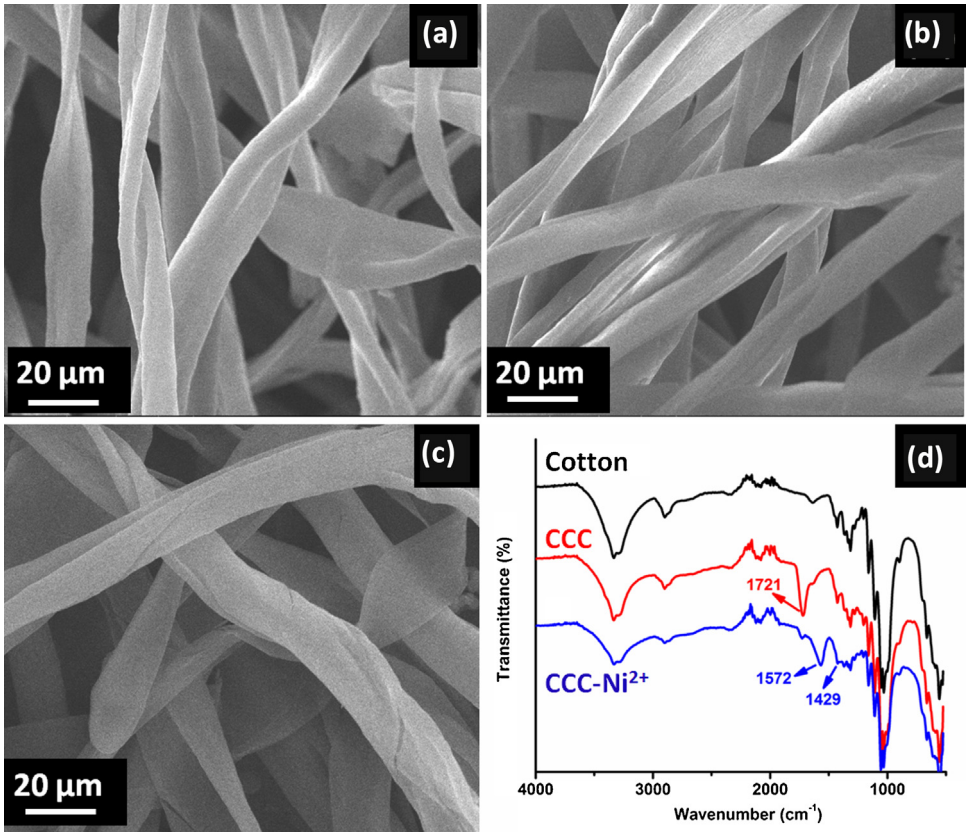

Fig. 2. SEM images of (a) cotton, (b) CCC, and (c) CCC-Ni^{2+} fibers; (d) IR spectra of cotton, CCC and CCC-Ni^{2+} fibers.

prepare two kinds of high specific adsorbents for biological analysis [23]. Very recently, carboxyl cotton chelator (CCC) with two free carboxyl groups on each structure unit was also exploited as IMAC support for the binding of metal ions in our work [24]. A novel CCC-Ti^{4+} fibrous material with high titanium content was prepared and subsequently proved to be a good adsorbent for phosphoproteomics analysis. Moreover, it is worth developing more CCC-based IMAC adsorbents due to its excellent binding capacity for metal ions and good biological compatibility.

In this study, a novel CCC-Ni^{2+} fibrous adsorbent was successfully prepared with a simple method on the basis of the coordination effect between Ni^{2+} and carboxyl group. Due to the high affinity of Ni^{2+} to 6 × His on His-tagged proteins, CCC-Ni^{2+} fibers were applied for selective enrichment of His-tagged protein from *E. coli* cell lysates using the lab-in-syringe format.

2. Experimental section

2.1. Reagents and materials

Citric acid, aqueous ammonia solution ($NH_3 \cdot H_2O$, 25 wt%), tris (hydromethyl) aminomethane (Tris), hydrochloric acid (HCl), sodium chloride (NaCl) and nickel(II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$, 98%) were all of analytical grade and purchased

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