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Facile fabrication of nickel immobilized on magnetic nanoparticles as an efficient affinity adsorbent for purification of his-tagged protein

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ABSTRACT

In the present research, an efficient, convenient, and inexpensive method for the one-pot synthesis of Fe_3O_4@Histidine is developed. Histidine is readily loaded on magnetic nanoparticles by one step and simple method without any supplemental linkers. In the structure of Fe_3O_4@Histidine, histidine covalently immobilized on the surface of Fe_3O_4, magnetic nanoparticles are able to trap Ni^{2+} ions through a strong interaction between nickel and histidines in protein tag. Two coordination sites of nickel are occupied with ligand on the surface of magnetic nanoparticles and four coordination sites have been remained that these sites will be occupied with histidine tag of recombinant protein A. The functionalized nanoparticles were spherical and well separated with an average diameter around 30 nm. The obtained magnetic nanoparticles have a saturation magnetization of about 54 emu/g. Fe_3O_4@Histidine-Ni was used to enrich and purify 6 × histidine-tagged recombinant protein-A directly from the mixture of lysed cells. It has been found that Ni(II)-immobilized Fe_3O_4@Histidine magnetic nanoparticles present negligible nonspecific protein adsorption and high His-tag protein binding capacity The average binding capacity (MW 42 k Da), is 700 ± 25 $\mu g \cdot mg^{-1}$ (protein/Fe_3O_4@Histidine-Ni).

1. Introduction

The improvement of approaches with efficient and simple strategies in order to separation of target proteins from cell extracts is very crucial topic in the area of proteomics. Now a day, in order to facilitate the protein purification, many target proteins are usually expressed with a tag [1,2]. Purification of recombinant proteins with engineered histidine affinity handles attached to the N- or C-terminus are desired [3]. Immobilized metal affinity chromatography (IMAC) has been considered as one of the most effective approaches for the separation and purification of His-tagged proteins from a matrix containing other undesirable biological elements. This process is based on the interaction between a transition metal ion (Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) immobilized on a matrix and the histidine exposed on the surface of the protein [4,5]. Generally, iminodiacetic acid (IDA), tris(carboxymethyl) ethylenediamine (TED), and nitrilotriacetic acid (NTA) can be used as metal-chelating agents [6,7]. Although this method is easily adaptable to any protein expression system, it has some limitations including the need for pretreatment to remove the cell debris and colloid contaminants, a relatively long operation time [8,9]. To overcome these limitations, some alternative nano magnetic adsorbents have been extensively investigated owing to their unique and superior physical and chemical properties: such as: (1) the nanometer engineered magnetic nanoparticles can faster move and easier enter into cells, (2) their high surface/volume ratio provide a higher binding rate and greater adsorption capacity, and (3) superparamagnetic property of magnetic nanoparticles prevented aggregation in the absence of external magnet and enabled them to redisperse in the solution rapidly, [10–12] therefore magnetic nanoparticles are widely used in various fields of biotechnology and biomedicine especially in purification and separation, for example, Zhang and co-workers showed the synthesis of Fe_3O_4 magnetic nanoparticles immobilized with iminodiacetic acid (IDA) that complexed by Cu^{2+} ions as IMAC adsorbents for selective capture of bovine hemoglobin (BHb) [13]. Feng and et al. reported the synthesis of superparamagnetic matrix (Fe_3O_4/SiO_2-GPTMS-Asp-Co) for purification of 6-Histidine-tagged proteins from the crude bacterial lysate [14]. Xu and co-workers reported the synthesis of nickel-nitrilotriacetic acid (Ni-NTA)-terminated magnetic nano particles (MNPs) for selectively polyhistidine-tagged

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protein capturing directly from cell extracts [15]. Zou and co-workers reported the synthesis of ferroferric oxide/L-cysteine (Fe_3O_4/Cys) nanospheres to separate His-tagged proteins from the mixed-protein solution, the binding capacity of His-tagged TRX protein was 48.2 $mg \cdot g^{-1}$ [16]. Wang and co-workers synthesized nanostructured Ni-NTA functionalized magnetic nanoparticle for a selective adsorption of his-tagged enzyme the nanobiocatalysts reached a specific loading capacity of 146 mg protein/mg MNPs [17]. Zhang et al. reported the synthesis of uniform magnetic core/shell microspheres functionalized with Ni^{2+}-iminodiacetic acid by one step purification and immobilization of his-tagged enzymes. It was determined the binding capacity of Ni-MNPs-3 to be around 103 mg/g (protein/beads) [18]. Zhang and co-workers reported the synthesis of Fe3O4/Au–ANTA–Co^{2+} nanoparticles to separate His-tagged proteins from the mixed-protein solution. The binding capacity for His-tagged SSA1 is 74 μg/mg [19].

In this work, we report a simple and facile method to prepare magnetic nanoparticles Fe_3O_4-Histidine-Ni *via* a one-pot method. These nanoparticles present high magnetic strength for the convenient and high-throughput detection of 6 × Histidine-tagged recombinant protein-A from the mixture of lysed cells with exposed Ni^{2+} sites on the surface of magnetic nanoparticles.

2. Experimental

2.1. Chemicals

The chemicals used in this work were obtained from Fluka and Merck and were used without purification. FT-IR spectra were recorded as KBr pellets on a Perkin-Elmer 781 spectrophotometer and an Impact 400 Nicolet FT-IR spectrophotometer. X-ray diffraction (XRD) pattern of the as-synthesized material was obtained using a Holland Philips Xpert X-ray powder diffraction (XRD) diffractometer (CuK, radiation, λ = 0.154056 nm), at a scanning speed of 2°/min from 10° to 100° (2θ). The content of nickel was determined by VISTA-PRO, CCD simultaneous ICP analyzer. Thermogravimetric/differential thermal analysis (TG/DTA) was performed on a Thermal Analyzer with a heating rate of 20 °C min^{-1} over a temperature range of 25–1100 °C under flowing compressed N_2.

2.2. Synthesis of Fe_3O_4@Histidine nanoparticles

5 mmol $FeCl_3 \cdot 6H_2O$ and 2.5 mmol $FeCl_2 \cdot 4H_2O$ salts were dissolved in 100 mL deionized water under vigorous stirring, then 2 mmol of Histidine and NH_4OH solution (25%, w/w, 30 mL) were added to the above mixture until the pH was raised to 11, till a black suspension was formed. This suspension was then refluxed at 90 °C for 6 h, with vigorous stirring. Fe_3O_4@Histidine nanoparticles were separated from the aqueous solution by magnetic decantation, before being dried in an oven overnight it should be washed with water and ethanol several times.

2.3. Preparation of Fe_3O_4@Histidine-Ni

For the preparation of Ni(II)-immobilized Fe_3O_4@Histidine magnetic nanoparticles, firstly, Fe_3O_4-Histidine nanoparticles (0.5 g) were ultrasonically dispersed in 100 mL of ethanol to form a homogeneous dispersion, and then loaded into a round-bottomed flask. $Ni(OAc)_2.4H_2O$ (0.24 g, 2 mmol) was added to this solution. The mixture was refluxed for 12 h. After stirring, the catalyst was harvested by aid of magnet, washed several times with ethanol to remove unreacted $Ni(OAc)_2$, and dried under vacuum at 50 °C.

2.4. Recombinant protein to be purified

Protein-A with 6 × histidine residues (His-tag) in their C-terminus, with molecular weights of about 42 KD, was chosen to be separated

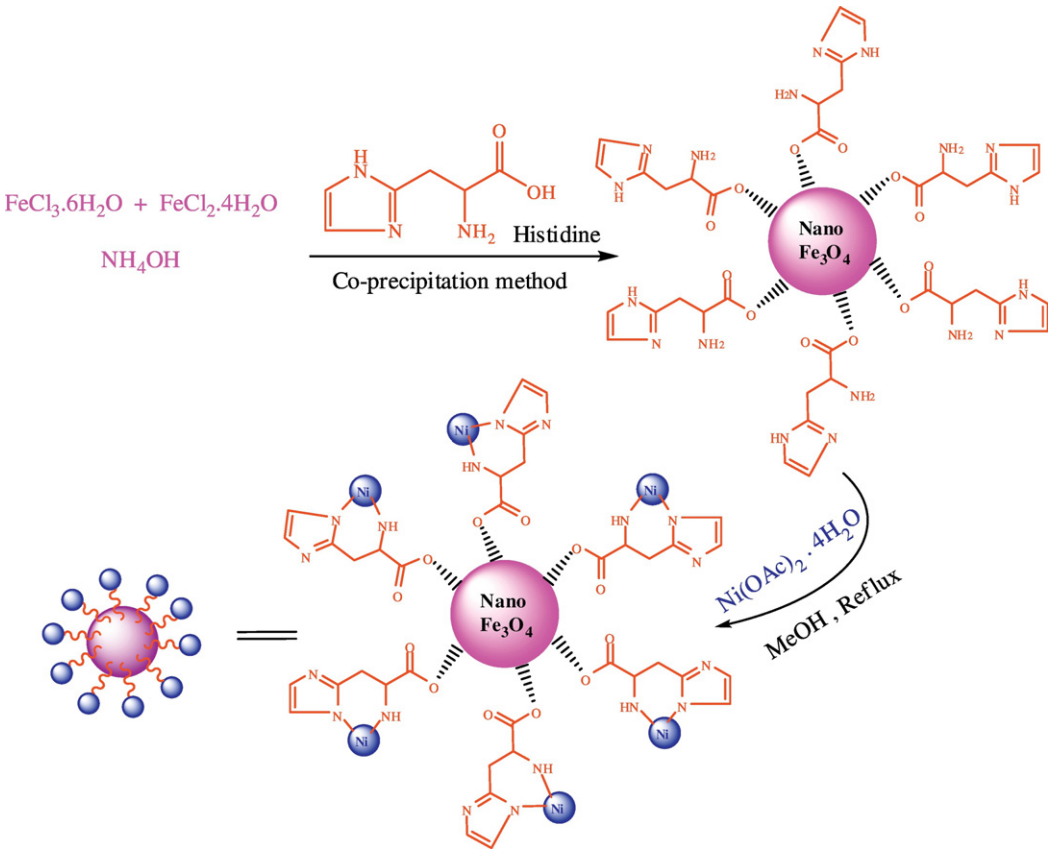

Fig. 1. Fabrication steps of Fe_3O_4-Histidine-Ni as a result of reaction between ferric salts and Histidine in facile one-pot co-precipitation route.