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Improving the binding capacity of Ni^{2+} decorated porous magnetic silica spheres for histidine-rich protein separation

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ABSTRACT

Biomagnetic immobilization of histidine-rich proteins based on the single-step affinity adsorption of transition metal ions continues to be a suitable practice as a cost effective and a up scaled alternative to the to multiple-step chromatographic separations. In our previous work [12], we synthesised Porous Magnetic silica (PMS) spheres by one-step hydrothermal-assisted modified-stöber method. The obtained spheres were decorated with Ni^{2+} and Co^{2+}, and evaluated for the capture of a H6-Tagged green fluorescence protein (GFP-H6) protein. The binding capacity of the obtained spheres was found to be slightly higher in the case Ni^{2+} decorated PMS spheres (PMSNi). However, comparing with commercial products, the binding capacity was found to be lower than the expected. In this way, the present work is an attempt to improve the binding capacity of PMSNi to histidine-rich proteins. We find that increasing the amount of Ni^{2+} onto the surface of the PMS spheres leads to an increment of the binding capacity to GFP-H6 by a factor of two. On the other hand, we explore how the size of histidine-rich protein can affect the binding capacity comparing the results of the GFP-6H to those of the His-tagged α-galactosidase (α-GLA). Finally, we demonstrate that the optimization of the magnetophoresis parameters during washing and eluting steps can lead to an additional improvement of the binding capacity.

1. Introduction

Superparamagnetic nanoparticles inserted in a non-magnetic matrix are used in numerous biological applications. When designing such composites, the main aim has been mostly to immobilize a bioactive substance on the surface with a sufficiently strong bond to ensure the composites stability prior to and during the application [1,2]. These properties make these composites excellent supports for the immobilization of enzymes and promising tools for proteins purification. Histidine-rich peptides (often H6) are probably the most used protein purification tags. Being short sequences, His-tags do not add significant metabolic load to the protein production process and they can be easily incorporated to the protein by simple genetic engineering at the upstream level [3].

In this way, immobilized metal affinity chromatography [4] is a separation principle based on the differential and reversible affinity of transition metal ions such as Zn^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+} for histidines [5], due to the coordination bonds formed between metal ions and amino acid side chains exposed on the protein surface. Electron-donor atoms (N, S, O) present in the chelating compounds of the chromatographic support are capable of coordinating metal ions and forming metal chelates, which can be bidentate, tridentate, etc., depending on the number of occupied coordination bonds [6].

Nickel nitrilotriacetic acid (Ni-NTA) beads have been most popularly used to purify proteins with polyhistidine affinity tag (His-Tag) [7]. This method employs an NTA-attached polymer resin to immobilize nickel ions (Ni^{2+}) and thereby separates His-tagged proteins through coordination chemistry. However, the conventional Ni-NTA system has some limitations, including the need for pre-treatment to remove the cell debris and colloid contaminants, a relatively time-consuming operation, and protein solubility. Several magnetic separation systems based on magnetic nanomaterials have been reported in an effort to go beyond the limitations of the Ni-NTA system. Xu and co-workers synthesized NTA-modified magnetic nanocrystals of FePt and Co/Fe_2O_3 and demonstrated their ability to separate His-tagged proteins [8,9].

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Recent work has reported on a novel magnetically recyclable protein separation system using magnetic nanocomposite spheres decorated with NiO nanoparticles for the purification of His-tagged GFP protein [10]. In this work, the synthesis process of the magnetic nanocomposite spheres consisted in different steps making the process significantly laborious. Other work has reported on Zn decorated core–shell magnetic particles for a reversible adsorption of BSA on silica maghemite shell–core composites. The authors claim that a minimum of 2 nm thickness of amorphous silica was necessary to induce the adsorption of the Zn^{2+} ions on the surface of the nanocomposite [11].

In our previous work [12], we reported on the design and synthesis of Porous Magnetic Silica (PMS) spheres by a simple one-step hydrothermal assisted modified-Stöber method. The porous morphology of the obtained spheres allows the homogeneous incorporation of the Ni^{2+} and Co^{2+} to the surface of the spheres and therefore their use in the capturation of histidine-tagged GFP (GFP-H6) protein. The obtained results of the binding capacity were found to be lower than the expected. For this reason, in the present work, an attempt to improve the binding capacity is presented focussing on the Ni^{2+} decorated PMS spheres. We demonstrate that increasing the amount of Ni^{2+} on the surface of the PMS spheres leads to an increment of 100% of the binding capacity to GFP-6H. On the other hand, we explore how the size of histidine-rich protein can affect the binding capacity comparing the binding capacity of the PMSNi spheres to His-tagged α-galactosidase (α-GLA) and to GFP-6H. Finally, we demonstrate that the optimization of the separation times and the concentrations of the suspensions during the washing and eluting processes can play a significant role in the improvement of the binding capacity.

2. Materials and methods

2.1. Synthesis of the Ni^{2+} decorated porous magnetic silica (PMS) spheres

The Ni^{2+} decorated porous magnetic silica (PMSNi) were synthesised according our previous works [12,13]. Briefly, as a first step, the γ-Fe_2O_3 nanoparticles were co-precipitated from an aqueous mixture of $FeSO_4$ ($FeSO_4{\cdot}7H_2O$) and $FeCl_3$ ($FeCl_3$). NH_4OH was used as a precipitation agent. The nanoparticles were dried at 60 °C for 24 h.

In the second step, the PMS spheres were prepared by the method of Stöber et al. [14]. For this, 14 mL of aqueous solution of cetyltrimethylammonium bromide (CTAB) 0.8 wt% and 4.5 mL of ammonium solution were mixed with 60 mL of ethanol containing 15 mg of NPs at 25 °C and pH of 10. After stirring for 5 min, 800 μL of tetraethyl orthosilicate (TEOS) was slowly added to the solution. The mixture was maintained under shaking bath overnight. The obtained suspension was transferred to an autoclave and was hydrothermally treated at 120 °C for 48 h. The resulting solid material was collected by magnetic separation and washed with distilled water. The dried powder was calcined at 400 °C during 8 h.

Finally, for the adsorption of Ni on the PMS spheres, 40 mg of the dried PMS spheres were suspended in 15 ml of distilled water and the suspension was shacked for 5 min. The required amount of $NiCl_2$ was dissolved in 10 ml of distilled water and then mixed with the prepared suspension of PMS spheres. The pH of the suspension was maintained at 9 during the process. The mixture was gently shacked during 4 h at room temperature and then the modified PMS spheres were magnetically separated and washed several times to remove the excess of the $NiCl_2$. The amounts of $NiCl_2$ were 4 mg and 40 mg for the samples PMSNi and PMSNi1, respectively.

2.2. Binding procedure of GFP-H6 and α-GLA proteins to the PMSNi and PMSNi1 spheres

The GFP-6H was provided by the Protein Production Platform (CIBER-BBN) (http://bbn.ciber-bbn.es/programas/plataformas/equipamiento). The production and purification details of His-tagged α-galactosidase (α-GLA) are described by Corchero et al. in reference [15].

Two-milligram of resuspended magnetic beads were washed and equilibrated with binding/washing buffer (50 mM Naphsophate, pH 8.0, 300 mM NaCl, 0.01% Tween®-20) and resuspended in 700 μL of the same buffer. The samples were incubated with the required previously purified protein (40 μg protein/mg

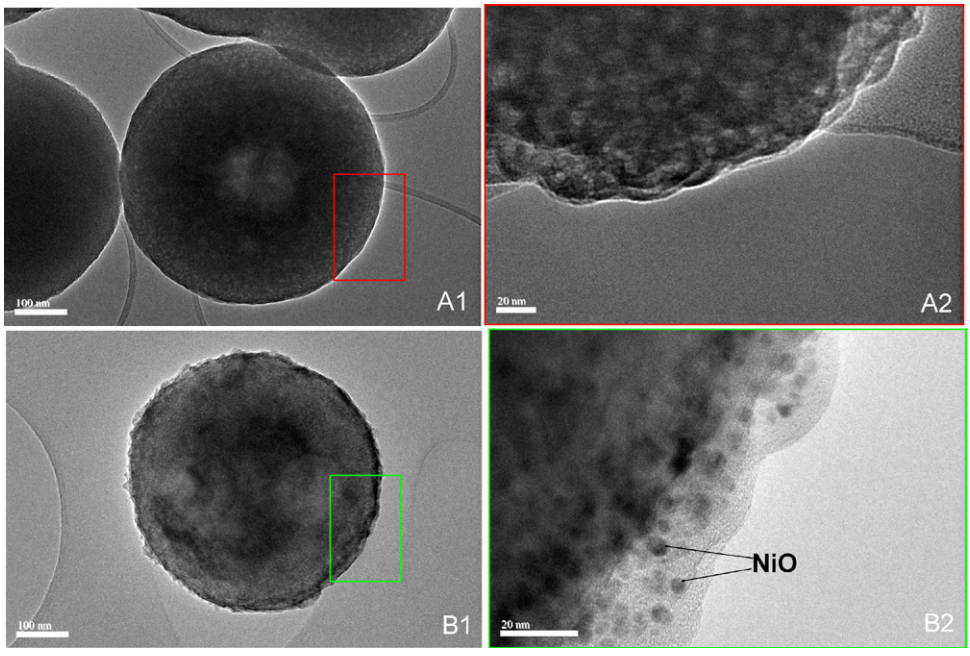

Fig. 1. TEM images of the obtained PMS spheres (A1, A2). The particles show a "hollow-like" morphology and a spherical shape with a diameter of about 400 nm. The particles are porous with disordered pores. B1 and B2 TEM images confirm that the obtained spheres were successfully Ni^{2+} decorated.