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Synthesis and optimization of nickel-iron nano-composite for GRP-78 immunoglobulin purification

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ABSTRACT

Protein separation and purification are widely used in all fields of life sciences and biotechnology. The development of new techniques that can replace current common methods increases efficiency and reduces production costs. Magnetic separation is one of the fast and easy methods for separating proteins, cells, and various molecules from the initial raw sample. In the present study, Ni-Fe magnetic nano-composite was synthesized and applicate for separation and collection of recombinant GRP-78 immunoglobulin protein. In this study, Ni-Fe nanocomposites are prepared by oxidation and reduction method. Then, the synthesized nanocomposites in each method are examined by different imaging and spectroscopy methods to measure the size of the nanocomposite and its surface morphology of Nano-composite, the crystal structure, and its stability. In the final stage, these nanocomposites are exposed to bind to protein histidine tag, and then the protein is collected, concentrated, and purified with an external magnet. In this study, the effect of independent variables including protein concentration, solution pH, and reaction time was simultaneously investigated on the dependent variable (size, protein loading) of iron/nickel oxide nanoparticles prepared by precipitation method. Simultaneous study of variables allows for more complete information on how different factors affect the dependent variable. The present study, the results of the morphology of nanoparticles showed that Ni-Fe Nano-composite had a size smaller than 100 nm and their shape is spherical. Vibrational Magnetometry indicated that the nanocomposite has good superparamagnetic properties and is well attracted by a permanent magnet. Therefore, it is suitable for collecting and purifying proteins from solution. Also, FTIR showed that nickel is abundant on the surface of the nanocomposite and is available for binding to the histidine tag of the protein. Continuing the research, protein loading on Ni-Fe nanocomposites was Optimization for several parameters such as time, temperature, pH, and concentration. Nano-composite can be loaded protein in a wide range of pH from 5.8 to 8.0. The protein loading rate by Ni-Fe Nano-composite is 20 minutes. Temperature analysis also did not show a significant difference in the loading rate. Finally, the evaluation of the efficiency of Iron-Nickel Nano-composite for protein adsorption under optimal conditions indicated the efficiency and ability of the nanocomposite to adsorb a high percentage of protein. The results indicate that Iron-Nickel Nano-composite can be used as an efficient alternative in the purification of a high percentage of protein. Magnetic affinity and ion exchange separation have been successfully used in various fields such as molecular biology, biochemistry, analytical chemistry, environmental chemistry, etc.

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1. Introduction

The separation and purification of proteins is widely used in all fields of life sciences and biotechnology, and in all research processes in the fields of vaccines, antibodies, antigens and markers, and recombinant drugs based on prokaryotic and eukaryotic systems, the separation, concentration, and purification of the recombinant product is essential, vital, and inevitable [1-2]. In the field of biotechnology, protein purification is performed by methods such as chromatography, electrophoresis, and ultrafiltration precipitation, with affinity chromatography being one of the most important methods [2]. This technique is currently considered the most powerful method available in downstream processes due to its high selectivity and recovery ability [3]. However, one of the disadvantages of all liquid chromatography columns is the inability of these columns to purify at high volumes in the presence of particles. As a result, these columns are not suitable for use in the initial stages of purification, where suspended solid particles and precipitated components are present in the system [2]. In addition, protein separation using chromatographic columns is very time-consuming and expensive, and it imposes very high stress on the proteins, which can destroy some of them and ultimately reduce operational efficiency. However, separation using magnetic particles, in addition to eliminating the stress and preventing the breakdown of large proteins, reduces costs and consequently increases efficiency [4]. Magnetic separation is a very simple process with one operational step, such that all purification steps can be performed in a single step. In some cases, it is even possible to perform the cell disruption, protein separation, and purification steps in one step, thus shortening the purification process. Also, due to the magnetic nature of the magnetic adsorbents and the non-magnetic nature of the remaining components, the target molecule is easily and selectively separated from the solution [2]. The absorption of electromagnetic waves in the MHz to GHz range has many applications, including electromagnetic pollution control, electromagnetic interference shielding, cloaking technology, and novel therapeutic applications [5]. Magnetic iron oxide-based nanocomposites have been interesting compounds for a wide range of applications in various environmental, energy, industrial, and medical fields. The formation of magnetic iron oxide-based nanocomposites is diverse and tunable. Magnetic iron oxide nanoparticles and nanocomposites have unique chemical and physical properties [6]. Magnetic materials can be classified into three major types, including maghemite (γ-Fe_2O_3), magnetite (Fe_3O_4), and hematite (α-Fe_2O_3). These classifications are useful for describing the advantages and disadvantages of materials when used in specific applications. For example, magnetite materials are commonly used in medical applications as drug carriers because they have the highest magnetic saturation properties compared to others (approximately more than 250 times) [7]. Magnetic nanoparticles and nanocomposites have found widespread applications not only in biomedical fields such as targeted drug delivery, magnetic resonance imaging (MRI), and biosensing, but also as highly efficient and recyclable heterogeneous catalysts in various organic transformations, owing to their large surface area, chemical stability, easy magnetic separation, and excellent reusability [8, 9]. The absorption properties of electromagnetic absorbing metal nanocomposites are better than those of pure metals or metal oxides. Among various nano-alloys, iron-nickel nano-alloys have been widely used as electromagnetic wave absorbers, magnetic sensors, antennas, catalysts, and magnetic stabilization systems due to their stable structure and excellent electromagnetic properties as well as high magnetic permeability [10]. Using nanocomposites instead of nanoparticles allows the properties of two or more materials to be used together, for example, using iron oxide-based nanocomposites allows for easy recovery after adsorption, in addition to adsorption and biocompatibility, due to the magnetic properties of iron nanoparticles [11]. In the present study, various methods of synthesis and coating of magnetic nanocomposites containing nickel-iron were investigated and their application in protein separation was considered to be used for the collection of recombinant immunoglobulin GRP-78 protein. Immunoglobulin GRP-78 is one of the best-known molecular chaperones (HSP70), endoplasmic reticulum, and a member of the 70-kDa heat shock protein family [12, 13]. GPR78 is induced on cancer cells and other cells under the influence of environmental stress and hypoxia, and this molecule prevents cell apoptosis by disrupting the function and homeostasis of the endoplasmic reticulum [14-17].

1.1. Objectives

In the present study, Ni-Fe nano-composite was synthesized and investigated for application in protein purification for collection of recombinant immunoglobulin protein GRP-78.

2. Materials and Methods

2.1. Material

$FeCl_2 \cdot 7H_2O$ (99.0%), $FeCl_3 \cdot 6H_2O$ (99.1%), $NiCl_2 \cdot 7H_2O$ (99.0%), 1-(4-chlorophenyl) imidazole all of them were purchased from Sigma–Aldrich (Steinheim, Germany). dihydrogen phosphate (KH_2PO_4) and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck. The solutions were prepared in deionized double distilled water (OES-SDLL-20L, USA) and all experiments were carried out at room

temperature. Recombinant immunoglobulin protein GRP-78 was obtained from Medical Sciences Research Center, Baqiyatallah University of Medical Sciences, and Tehran, Iran.

2.2. Apparatus

UV-Vis spectroscopy was performed by Cary spectrophotometer (100 Bio-model, USA). Fourier transform infrared (FTIR) spectra were recorded using Fourier transform infrared spectrometer (Model Nexus 870, Thermo Nicolet Co. USA). Scanning electron microscopic (SEM) images were obtained using a Scanning electron microscope (Model LEO 440i, UK).

3. Results and Discussion

3.1. Synthesis of Ni-Fe nanocomposite

The iron oxide nanoparticles were prepared by co-precipitation of Fe (II) and Fe (III) in an alkaline environment. In short, 5.4 g $FeCl_2 \cdot 7H_2O$ and 2.0 g $FeCl_3 \cdot 6H_2O$ were dissolved in 33 ml of 10 mM HCl and 167 ml of 1.5 M NaOH was added drop wisely to the solution under vigorous stirring. A black precipitate immediately formed. It was collected with a permanent magnet and washed with distilled water to remove of excess NaOH, and then it heated in 60 °C to drying. To synthesize the iron-nickel magnetic nanocomposite, nickel chloride (II) was replaced instead of iron chloride (II) in the nanoparticle structure [18, 19]. In this way, the ability to attach histidine tag of protein to magnetic nanoparticles was provided.

3.2. Purification of immunoglobulin GRP-78 protein by Ni-Fe nano-composite

Ni-Fe nano-composite and immunoglobulin GRP-78 protein were placed in proximity, and the histidine tag of the protein was attached to the magnetic nickel nanocomposite. The proximity time was 20 min.

Then, the protein-bound iron-nickel bio-nanocomposite was separated from the rest of the solution with a permanent magnet, washed with buffer containing imidazole (250 mM), and prepared for measurement of protein concentration by UV-Vis spectroscopy.

3.3. Evaluation of Ni-Fe Nano-composite

3.3.1. Field Electron Microscopy (FESEM)

The microstructure, morphology and average size of the nanoparticles were investigated using a field emission scanning electron microscope. Iron oxide nanoparticles (A) and Ni-Fe Nano-composite (B) were evaluated by scanning electron microscopy, and as can be seen in Fig. 1 Supp, the size of these particles is below 100 nm and is spherical in shape, but the Ni-Fe Nanocomposite are slightly larger in size, which is expected given the periodic table and the larger radius of nickel compared to iron. Both images show that the nanoparticles are not agglomerated and have a uniform size.

3.3.2. Infrared spectroscopy

Iron oxide nanoparticles have a peak in the range of 3500-3000 cm^{-1} that is related to the stretching of the O-H bond and also the Fe-O group has a characteristic stretching absorption in the region of 580 cm^{-1}. Also, the bending band of the Fe-O bond is seen at 1363 cm^{-1} (Fig. 2 Supp-A). As can be seen in Fig. 2 Supp-B, in Ni-Fe Nanocomposite, these peaks have significant changes. A strong peak has appeared in the region of 783 cm^{-1} that is related to the bending absorption of the Ni-Fe bond [20-23]. This experiment shows that nickel ions have successfully replaced iron oxide in the nanocomposite.

3.4. Vibrational Magnetometry (VSM)

Another experiment that was conducted to confirm the formation of magnetic nanoparticles of Ni-Fe Nano-composite was the hysteresis test. In order to investigate the magnetic properties of the prepared composite, a variable gradient force magnetometer was used to record the magnetic remanence loop. By placing the sample under an external magnetic field generated in the magnetometer, the flux density or magnetic induction (B) in it changes as a function of the magnetic field intensity (H).

With increasing magnetic field intensity, the magnetic flux in the material increases. In diamagnetic materials, the increase is linear. In paramagnetic and diamagnetic materials, it increases until it reaches a constant value that is saturated (Bs) and its corresponding magnetization is (Ms). In the saturated state, all magnetic dipoles are aligned with the external field. By reducing the magnetic field intensity to zero, the magnetization curve does not return from its original path and at H = 0, the magnetic flux density does not become zero. In other words, even though the magnetic driving force has been removed from the sample, the sample retains some magnetic property. This magnetic property that appears after the magnetic field is removed is called remanence or residual magnetism.

The unit of magnetic field strength in the cgs system is the oersted (Qe). Magnetic flux density (B) is the intensity of the magnetic field inside a material subjected to a field H and is the number of magnetic field lines per unit area inside the material. Its unit in the SI system is the Tesla (T) and in the cgs system, the Gauss (G). Magnetization (M) is the sum of the magnetic moments of the material per unit volume (or per unit mass) and its unit in the cgs system is emu cm^{-3}

(or emu g^{-1}). emu is equal to one erg/G. Magnetization is expressed by Eq. 1:

$$B = \mu_0 H + \mu_0 M \quad (1)$$

μ_0 is the magnetic permeability of vacuum, which is a universal constant. The magnitude of the magnetization vector is proportional to the applied external field according to Eq. 2.

$$M = x_g H \quad (2)$$

x_g is the magnetic susceptibility of the mass, whose unit in cgs is Qe^{-1} emu g^{-1}. Figure 3 shows the residual loop of the desired nanocomposite. As can be seen in the figure, the nanocomposite exhibits superparamagnetic properties. The saturation magnetization of the composite was estimated to be 26 (emu/g) from the graph (Figure.1).

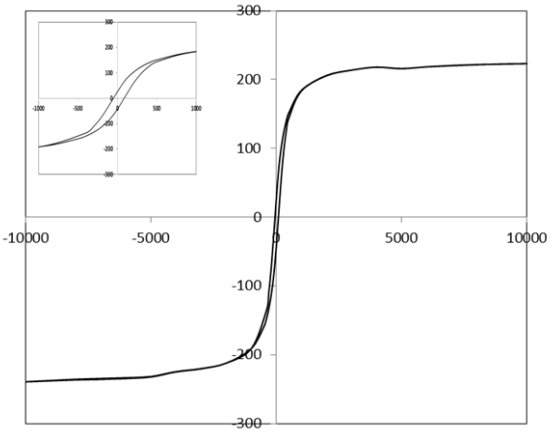

Fig. 1. Magnetic hysteresis loop of Ni-Fe Nano-composite measured at room temperature. The inset shows an expanded low-field curve.

3.5. Optimization of protein loading on Ni-Fe nanocomposites

To investigate the best protein loading rate by nanocomposite, the following variables were optimized: time, temperature, pH, and concentration.

3.6. Investigating the concentration of protein loading by Ni-Fe nanocomposite

Different concentrations of protein were placed in the vicinity of the Ni-Fe Nanocomposite to determine the highest concentration of protein that the Ni-Fe Nanocomposite was able to absorb and that concentration was used for optimization. As can be seen in curve 4, the highest protein loading rate by the nanocomposite (1 gr/mL) is 24 µg/mL (Figure 2).

3.7. Time Optimization

One of the important issues in the present study was to investigate the appropriate time for protein binding to the nanocomposite in order to determine the appropriate time for maximum protein binding to the nanocomposite. Therefore, times of ~1, 5, 10, 20, 30, 60 and 120 minutes were selected to place the protein in a shaker at room temperature in the vicinity of the nanoparticles. Then, the amount of purified protein was recorded using ultraviolet-infrared spectroscopy at a wavelength of 280 nm. It seems that 20 minutes is enough time for protein absorption. The concentration of Ni-Fe Nanocomposite and protein was 1 gr/mL and 24 µg/mL, respectively (Figure 3).

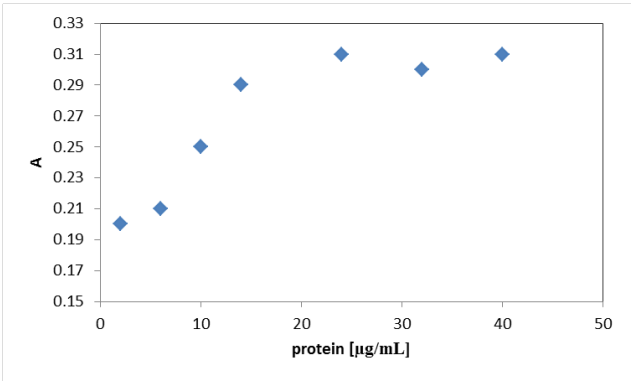

Fig. 2. Curve of immunoglobulin GRP-78 protein loading by Ni-Fe Nanocomposite

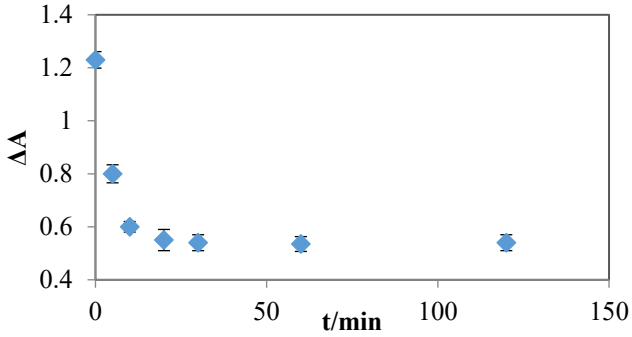

Fig. 3. Curve of protein adsorption changes on Ni-Fe nano-composite at different times

3.8. pH Optimization

At different pHs, the protein was placed in the vicinity of the Ni-Fe Nanocomposite, and the best absorption pHs at a wavelength of 260 nm were selected for Ni-Fe Nanocomposite optimization. The results showed that protein loading by nanoparticles can be performed in a wide range of pH. However, better binding appears to occur at acidic pHs. Therefore, the pH=6.6 was chosen as the optimal pH. The concentration of Ni-Fe Nanocomposite and protein was 1 gr/mL and 24 µg/mL, respectively (Figure 4).

3.9. Temperature Optimization

Under optimized time and concentration conditions, the sample were examined at temperatures of 25 and 15^0C protein absorbance was read at UV 280 nm. The

absorption intensity at each temperature was 0.79 and 0.77 respectively. The concentration of Ni-Fe Nanocomposite and protein was 1 gr/mL and 24 µg/mL, respectively.

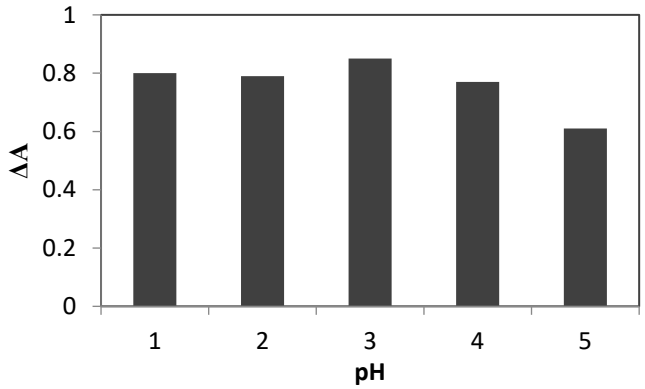

Fig. 4. the effect of different pHs on protein adsorption onto Ni-Fe Nanocomposite (1 to 5 are pHs 5.8, 6.6, 7, 7.4 and 8 respectively).

4. Conclusion

Protein separation and purification is widely used in all fields of life sciences and biotechnology. Magnetic separation is one of the fast and easy methods for separating proteins, cells and various molecules from the initial raw sample.

Traditional nickel chromatography columns (Ni-NTA Agarose), although still effective for high and medium scale, have some problems that have led to the expansion of research into the use of nickel magnetic nanoparticles (Ni-NPs) for protein purification, which have the following advantages: the washing and final washing steps are also much faster due to the elimination of centrifugation, excellent extraction efficiency due to the much higher specific surface area of the nanoparticles, which provides a greater number of binding sites, this is especially important for dilute samples. Nanoparticles may provide higher purity due to more effective washing. There is no sample loss due to column blockage.

There is no need for a column, support, peristaltic pump or centrifuge, so the extraction steps are simplified and also, no need for expensive FPLC/HPLC systems. In the present study, the chemical synthesis method of a magnetic nanocomposite containing nickel-iron was used in the separation and purification of recombinant immunoglobulin GRP-78 protein.

This Ni-Fe Nanocomposite was exposed to the protein to bind to its histidine tag, and then the protein was collected, concentrated and purified with an external magnet.

In this study, the optimization of temperature, time, pH and concentration was investigated to find the best conditions for protein binding to the nanocomposite, and it was found that room temperature and a time of 10 minutes at almost neutral pH can provide the optimal conditions, and under these conditions, approximately 44% of the protein is purified.

Supplementary files

Supplementary file 1.

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