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Synthetics of $NiFe_2O_4$ nanoparticles for recombinant His-tag protein purification

S.Imani[1,*]
A.M.Zand[1]
M.Saadati[1, 2]
H.Honnari[1]
B.Maddah[3]

[1]Department and Research Center of Biology, IHU, Tehran, Iran.
[2]Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[3]Department of chemistry, IHU, Tehran, Iran.

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ABSTRACT

Recombinant protein purification is a kind of sensitive and expensive method in genetics engineering. Genetic manipulation leads to the expression of various proteins; it should be isolated with high purity finally. Differed methods for protein purification are categorized, based on cast, quality, Easy work and side effect of protein. In this article, we are investigating his His-tag protein purification by magnetic nanoparticles.

$NiFe_2O_4$ nanoparticles were synthesized by Co-precipitation method, than was dissolved in 0.05 NaCl solutions. Tube containing of nanoparticles and buffer was located in magnetic field. Nanoparticles were separation by the three-stage washed. According to the protocols, nanoparticles located in his-tag protein and the end protein were predicated, and analyzed by the Electrophoreses.

The results of gel showed can be extracted that the proteins form a weak that the results of gel showed that proteins isolated are poor by this method. By supplementary study, we get new age or tree-age nanoparticles, that Ni is the surface of N.P. This age of nanoparticles is fit to protein purification.

Nano-particle synthesis in this article, are created a two-dimensional grid of nickel and iron oxide nanoparticles that this structure is not suitable for purification of His-tag protein. So the magnetic nanoparticles have a three-dimensional structure of the nickel nanoparticles on the surface of the exposed histidine to be enough space to connect and link. It is hoped in future studies with these types of nanoparticles synthesis to achieved His-protein isolation kit.

Keywords: *MNP; Recombinant protein; Protein purification*

INTRODUCTION

Quick and easy separation of protein products in the proteomics science and diagnosis is very important and vital. Histidine tag on recombinant proteins using is conventional methods for protein separation [1]. The use of nanoparticles in diagnosis and treatment are also widely studied and used [2].

* Corresponding author:
Saber Imani
Biology Department, Basic since Faculty, IHU, Tehran, Iran.
Tel +98 912 6407588
Fax +98 21 77104935
Email imani.saber@yahoo.com

We used new methods for extraction, isolation and identification of proteins. In these methods separate proteins from other biological products and proteins are separated. One of these methods is using magnetic nano particles (MNP) [3]. The greatest difference between these methods is how the link can be done between the protein products of the nano- particles. This difference in the characteristics of these products will determine their value and could lead to bio-molecule detection in different levels [3-4]. They have identified a number of metal ions that can bind with many proteins [5]. Amino acid is in some metal ions, atoms like sulfur, nitrogen, oxygen and has a high tendency to interact with the protein and so can be suitable factor for protein separation [5-6].

Many amino acids Such as Asparagine, Glutamine, Cysteine and Histidine that are considered are electron receptors. Amino acid histidine, an amino acid that has a tendency to bond to metals and more is known in metalloenzyme [6, 7]. Analysis of three-dimensional (3D) shows more metalloprotein that histidine is stable protein configuration by metal binding [8-9]. More proteins placed at the level Consecutive Histidine sequences compared with the single- Histidine sequences, which are located within the protein configuration, could be have a higher desire to have interactions with nanoparticles. For example, most sequence of poly - histidine tag used as a function in the molecular column for proteins separation. That is interacting with the Nickel. On the other hand, only the structure of natural proteins can have a 6 consecutive histidine sequence [10]. Among the metal ions, Ni^{+2} as one of the best combinations, which recently isolated peptide specific targets 6-His's-tag is, used it can be made of nickel metal [11]. To connect the metal ions to the magnetic nanoparticles are used for protein separation, a chemical intermediate used in the plant. Nitrilotriacetic Acid (NTA), Dopamine, Carbodiimide and some of these chemicals polymers are including compounds [11]. This is the intermediate compound Ni-NTA is to separate proteins and used with histidine tag sequence [1]. This method is used of NTA that linked to nickel polymer and protein-containing labels 6-9 histidine, attached to the nanoparticles are isolated and the purity of the environment [2]. Performance of magnetic nanoparticles, the interaction of the histidine tag purified proteins shown in Figure 1.

Link between magnetic nanoparticles and proteins tagged with Histidine is a covalence type. This link is very strong and stable and that is why it is not separated from the nickel during the separation of protein.

By creating covalent bands, is caused binding sites and position for three-dimensional protein separation until protein bonds to nickel ion in the surface of nanoparticle complex. The interaction of structure and form three-dimensional space creates a situation that is shown in Figure 2. If this does not seem to create a space that their performance is impaired.

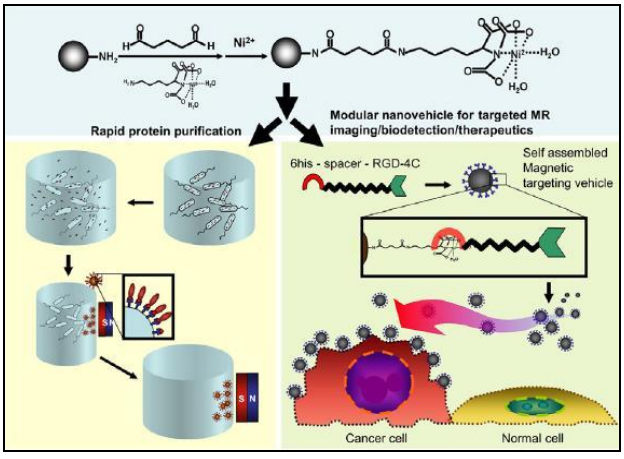

Fig.1. In this form is shown simplified procedures for the rapid uptake of histidine protein on the bacterial cell with magnetic nanoparticles. Nanoparticles can be mixed with a soluble bacterial lysis and finally with an external magnetic force to be separated, nanoparticles + solution containing nanoparticles of other proteins that lack the protein. The nanoparticles of the protein to be used again.

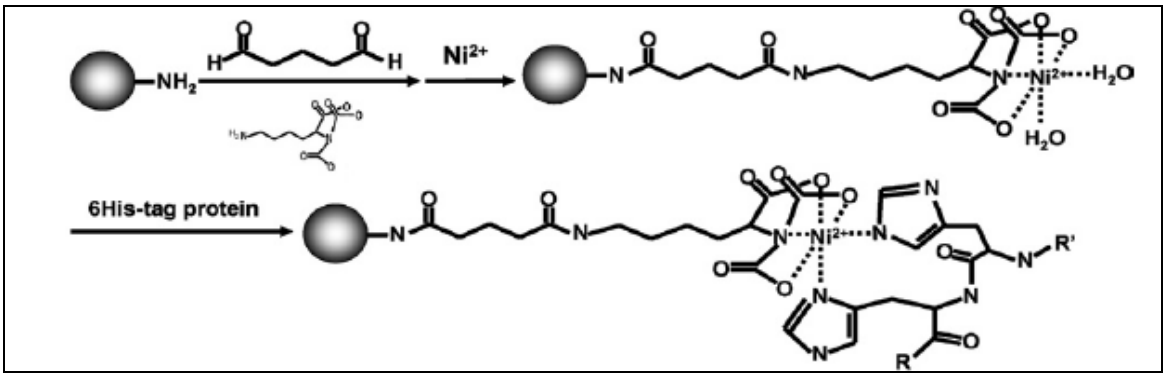

Fig.2. Surface changes in complex nanoparticles Fe_2O_4-NTA-Ni are stable covalent bonds between the amino acid, nanoparticles level and Glutaraldehyde. NTA by covalent band connected to the nanoparticle and the carboxyl group. With this connection, open the two binding sites for binding to 6 His-tag protein.

In this paper, the synthesis of magnetic nano-particles $NiFe_2O_3$ was evaluated efficiency and specificity for the isolation of recombinant proteins 6 or 9 histidine. SDS-PAGE analysis was performed to investigate the binding proteins to nanoparticles $NiFe_2O_4$ and the ability of Structure being evaluated by monoclonal antibodies in the Western blot test.

EXPERIMENTAL

Materials tested

In this study, all materials and chemical reagent such as the proteins were bio molecular. Iron (III) nitrate, ferric nitrate and nickel (II) nitrate from Sigma Co-America Aldrich, were prepared. All evaluation salts dissolved in two ionizing water. Salt solution HEPES, and urea imidazole the same company were ordered. Protein and monoclonal antibody evaluation is synthetic protein of 50 kDa that was prepared by Nanotechnology research center of *Baqiyatallah* University.

Synthesis of magnetic nano-particles

The MNP $NiFe_2O_4$ nanoparticles studied were synthesized using chemical co-precipitation method. First, 200mL of purified, deoxygenated water was bubbled by nitrogen gas for 30 min and the Ni $(NO_3)_2$ and Fe $(NO_3)_3$ salts with a molar ratio of 1:2 were successively dissolved in ultrapure water with vigorous mechanical stirring. Under the protection of nitrogen gas, the mixture was heated up to 70 ˚C in a water bath and then NaOH 2M was added drop-wise into the above solution till pH 11. After that, the stirrer was switched off and magnetic particles settled gradually. The precipitate was isolated by an external magnetic field and the supernatant was decanted. To obtain pure and neutral pH products, synthesized materials were rinsed again with ultrapure water and the rinse was discarded as before. The rinsing was repeated for a third time and the magnetic nanogel was then freeze-dried. Finally, magnetic nanoparticles such as $NiFe_2O_4$ were obtained.

Nanoparticle synthesis will improve and the reaction repeated with the sample average. The overall reaction is the synthesis of nanoparticles $NiFe_2O_4$ as follows:

$$2FeCl_3 + NiCl_2 + 8\,NaOH \rightarrow NiFe_2O_4 + 8NaCl + 4H_2O$$

Analytical methods of nanoparticle

To review the features of this nanoparticle dispersion was performed crystal X-ray (XRD). For nanoparticle size composite photo electron microscope (TEM) was used to set both of these analysis showed that the synthesis of nanoparticles in diameter of about 20±5 nm found. To check the size of the nanoparticle was taken termination electron microscopy (TEM) image. TEM and XED analysis showed that the synthesis of nanoparticles has a diameter of about 20± 5 nm.

Separation of proteins with magnetic nanoparticles $NiFe_2O_4$

For protein separation capabilities nanoparticle $NiFe_2O_4$ (based on previous studies), 500 mM protein His - tag or the test sample was

added to isolate high-quality samples done. On the other hand, The synthetic nanoparticles in a separate clean tube with distilled water and the solution was well-Vertex. Then 30 ml of nano-particle solution to the protein solution was beforehand prepared were added. For increase protein separation was used the greater the volume of nano-particles (50 µM).

Afterwards, the tube containing the sample and the nanoparticles are mixed gently without Vertex to 10 times and incubated at room temperature for 10 min to complete the interaction between proteins and nanoparticles. Tube containing the solution was put in the presence of an external magnetic force for 30 seconds till the samples containing Pr-$NiFe_2O_4$ absorbed to the external magnetic field. Then the tube is slowly removed from the magnet and 150 µl buffered connection including imidazole detergents, urea and HEPES are added to the sample tubes. The particles were collected and attached to the magnet by sampler slowly the above was repeated 3 times. Magnet and then removed from the tube and 100 µl of buffer amplifier and the solution was mixed. To increase the quality of work, you can change the buffer size is increased up to 200 µl. Finally, the solution incubated for 1-2 min at room temperature and for verification of work, was used of SDS-PAGE and Western blot.

RESULTS AND DISCUSSION

In this paper, a synthetic nanoparticle size is 5 ± 20 nm, the distribution of X-RAY, nanoparticle diameter is investigated and confirmed in Figures 3 and 4 also crystallographic structures of these nanoparticles can be observed.

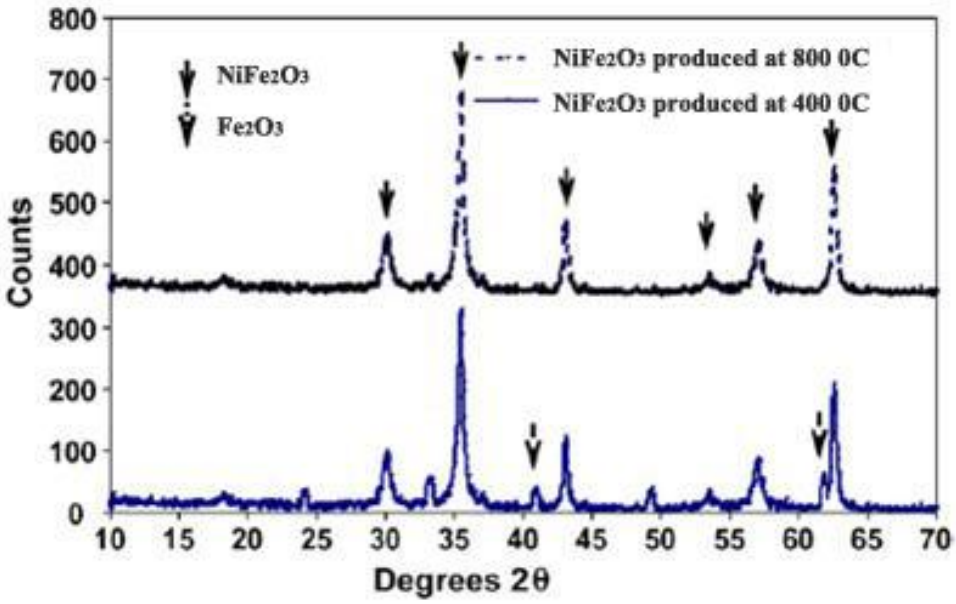

Fig.3. XRD diagram to check the size of nanoparticles synthesized nanoparticle synthesis

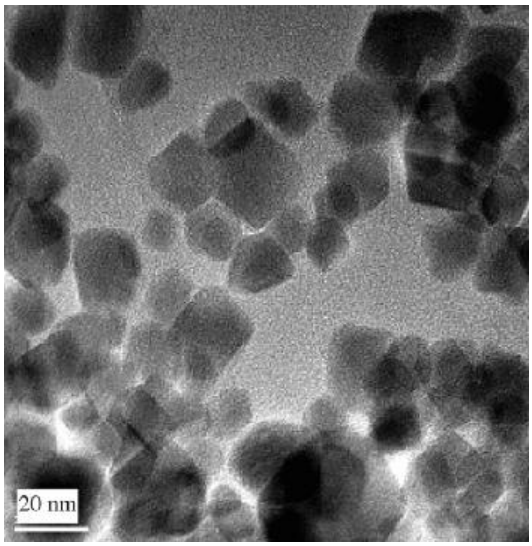

Fig.4. TEM nanoparticle shape NiFe2O4. Crystal structure in the shape of nanoparticles is visible

SDS-PAGE gels for control and test samples are shown in Figure 5. The initial study of the gel showing protein samples donor separation is the magnetic nanoparticles.

As is clear in this figure, the test is a weak band in the figure below shows a flash. 1 and 2 columns show that the buffers and materials used in this article has no negative impact on the protein. And in fact has been confirmed on the non-destructive method.

Figure 6 results western blot with specific antibodies against the protein studied, shows. Samples in the area between 40-50 k.Da with a weak bond is stronger in the next bond is evident in the control sample. Perform this test, the results of SDS-PAGE gel to confirm.

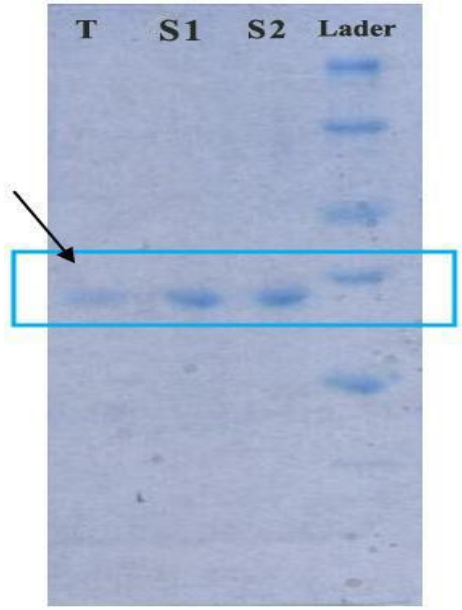

Fig.5. SDS-PAGE gel for a specific band of protein. Columns from left to right respectively are:
Column 1: ladder 120 kDa.
Column 2: control sample without nanoparticles containing only the original protein samples.
Column 3: control sample collected after the purification process and in the absence of nanoparticles.
Column 4: The amount of protein separated by nanoparticles.

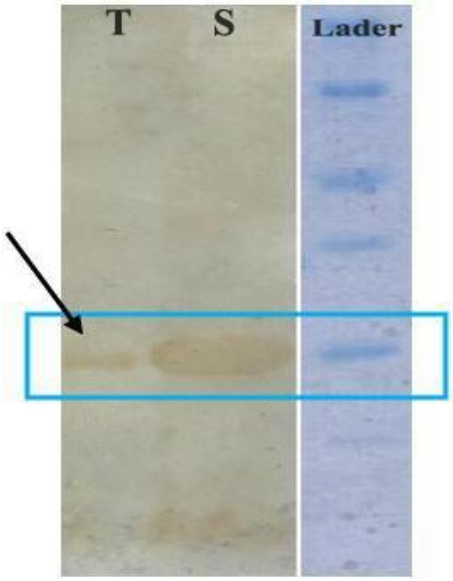

Fig.6. western blot test for samples. Columns from left to right respectively are:
Column 1: Ladder 120 kDa.
Column 2: control sample with their specific antibodies reacted.
Column 3: sample test with the specific antibody response is poor form.

In this article, magnetic nanoparticles $NiFe_2O_3$ (5 ± 20 nm) synthesized and its ability to isolate proteins Histidine were evaluated. That analysis to check the performance of nano-particles for protein separation was carried out showed that the separation with magnetic nanoparticles $NiFe_2O_3$ not performs at a high level of protein bands separated by a SDS-PAGE gel and blotting is western.

According to the results, we can say that design and construction of such nanoparticles with applications in three-dimensional structure should be considered. Distance between Ni and nano-particles in one of the main reasons for the rise is the ability to isolate nanoparticles. Furthermore, three-dimensional atomic structure and the type of interface between the graft and nickel also enhance the ability to link between nickel and protein can determine. Figure 2 shows that what the position and three-dimensional shape required for establishing the complex links covalent magnetic nanoparticles are more suitable for transplantation, and the separation of proteins can bind with higher power and accuracy are performed [13]. Chenjie Xu and colleagues of dopamine as intermediate between groups operating magnetic metal nanoparticles have used the protein separation performance has had about 57 percent [14] In fact, high specificity and efficiency of diagnostic kit protein using MNP connection depends on the position of metal ions such as nickel is [16-15].

Finally, the structure of the synthesized nano-particle separation ability is not too much protein for protein separation is not appropriate. Therefore recommended for synthesis of nanoparticles with three-dimensional structure of this type of application should be made the highest considerations. Moreover, transplantation must be specific and accurate.

CONCLUSION

According to the results of this research paper will prove that the separation of recombinant proteins with this method is not high performance. So in other studies to improve the method of intermediate or other polymers can isolate proteins with magnetic nanoparticles can be improved.

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