

# Immobilization of Enzymes on Functionalized Magnetic Nanoparticles for Efficient Biocatalysis

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## ABSTRACT

In this paper, iron oxide magnetic nanoparticles were synthesized by co-precipitation method and were functionalized with aldehyde group. Spherical superparamagnetic nanoparticles with diameter of 100 nm were obtained based on the results of field emission scanning electron microscopy (FESEM) and vibrating sample magnetometer (VSM). These particles were further applied for immobilization of enzymes by covalent binding between aldehyde group on particles and amino group on the enzyme surface. Different pH was applied for optimization of the immobilization conditions. The result showed that pH 4.0 is the best pH for immobilizing BSA on the nanometric support.

**Keywords:** magnetic nanoparticles, superparamagnetism, immobilization of enzymes, BSA, biocatalysis

## 1 INTRODUCTION

In recent years, nanotechnology draws increasing attention both from the public and from the academia. These nano-sized materials behave differently comparing with their bulk materials regarding to their chemical, physical, electrical [1] and optical properties [2]. In the pool of these nanometric materials, magnetic nanoparticles (MNPs) are really attractive due to its high surface area to volume ratio and special magnetic properties [3]. So far, MNPs have been applied for several bio-related applications, for instance, supports for bio-separation [4] and bio-adsorption of useful compounds, vehicles for immobilization and delivery of enzymes or drugs [5], heating reagent in hyperthermia, supporting material in biosensors [6], as well as contrast reagent for magnetic resonance imaging (MRI) [7]. Among them, the application of magnetic nanoparticles for immobilization of enzymes has great potential to be commercialized and applied in industry.

Research related to immobilization of enzymes started from the 1950s. The purpose of immobilization is to obtain better enzyme performance regarding to its stability and

activity during the catalytic process [8]. In general, there are several types of immobilization based on the physical and chemical interactions, most of which applied micrometric solid support or micro gel to attach or trap enzymes. Although enzymes could maintain good stability by these approaches, there are two critical problems occurred: (i) recycle and reuse of the immobilized enzyme become difficult when solid impurities exist in the catalytic system; (ii) there are tremendous mass transfer limitation for the substrate diffusing into the vicinity of the catalytic center of the immobilized enzyme. As a result, the yield of production by using immobilized enzyme is always restricted.

Recently, the applications of MNPs to attach enzymes have been proposed, which could effectively solve those above mentioned problems [9]. First, the magnetic material with enzyme immobilized on could be easily separated by applying external magnetic field. Thus, recycle and reuse of the immobilized enzyme could be simplified and be conducted for several cycles without influence the following process. The cost will be lowed down as well. Second, those nano-sized particles could maintain high dispersibility both in aqueous phase and organic phase, and provide huge functional surface area for immobilization of enzymes. The mass transfer limitation will no longer be a problem in this case.

In this study, BSA was chosen as a model enzyme. BSA belongs to non-redox protein, which is generally considered inert in biocatalysis. However, recently, Klivanov and his research group found that this protein could catalyze certain reactions with good stereoselectivity, including epoxidation of electron-deficient alkenes, oxidation of amines and reduction of ketones [10]. In our following work, the reason for choosing this protein is two-fold. First, the source of BSA is rather cheap and conventional, and the detecting method of this protein is also well-established. Second, BSA is a wide-studied model protein. It will offer great theoretical convenience for immobilization of other enzymes and provides good foundation for related research in biocatalysis.

## 2 MATERIALS AND METHODS

### 2.1 Materials

Ferric chloride hexahydrate, ferrous chloride tetrahydrate, potassium oleate, ethylenediamine, glutaraldehyde and ammonium hydroxide are all supplied by Sigma-Aldrich.

### 2.2 Methods

0.01 mol ferric chloride hexahydrate, 0.005 mol ferrous chloride tetrahydrate and 0.01 mol potassium oleate were dissolved in 100 ml deionized water. This reaction mixture was stirred by mechanical stirrer at 80 °C under argon protection. Then 0.074 mol ammonium hydroxide was added inside this system and iron oxide seeds were formed simultaneously with black color. Afterwards, 0.024 mol monomer and 0.00011 mol initiator were added inside 5ml of previous seeds solution. The polymerization was continued for 1 hour. The particles were purified by centrifugation and controlled by permanent magnet. Then the particles were treated by 0.1 M ethylenediamine for 6 hours in order to carry with amino group. Glutaraldehyde was further used to functionalize the particle with aldehyde group. The particles were freeze-dried and were used for further characterization.

Immobilization of BSA was conducted as follows: 2 mg BSA was mixed with 0.675 mg aldehyde functionalized particles in 1 ml 0.1 M citric acid buffer. Different pH value from 3.0 to 8.0 was used. The solution was shaken at 500 rpm at 25 °C for 12 hours. Then the particles were centrifuged and controlled by permanent magnet and washed with 0.4 ml deionized water twice. The concentration of BSA was measured by using Bradford reagent at 595 nm with negative controls.

## 3 RESULTS AND DISCUSSION

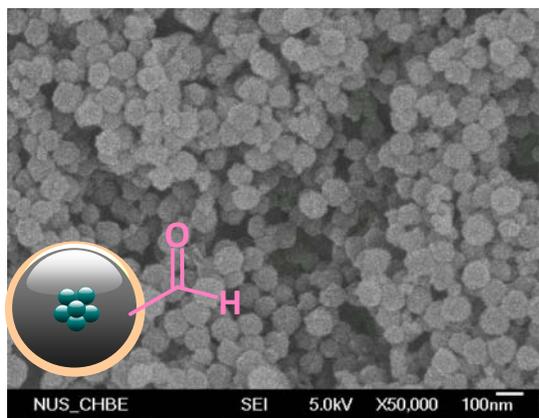


Figure 1: FESEM of aldehyde functionalized magnetic nanoparticles.

Figure 1 shows the FESEM results of the synthesized magnetic nanoparticles. The particles are spherical size and iron oxide seeds were trapped inside the polymer matrix.

From the figure, the mean size of the particle is 100 nm in diameter. The hydrodynamic size of BSA is around 8 nm from the dynamic light scattering (DLS) measurement results. So the size of the magnetic nanoparticles is suitable for immobilization of enzymes.

VSM was further used to measure the magnetic property of the particles. From the results in Figure 2, we found that these magnetic nanoparticles belong to superparamagnetic particles. However, the saturation magnetization is not high, which is only 1.69 emu/g. This may be due to the decrease of the anisotropy of the iron oxide surface after thick coating. Figure 2 exhibits the curve of VSM.

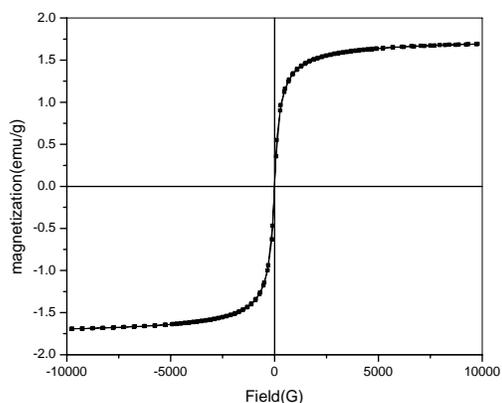


Figure 2: VSM of polymer coated magnetic nanoparticles.

After functionalization with aldehyde group, the particles were ready for immobilizing enzyme. Table 1 shows the results of immobilization at different pH.

pH	wash and supernatant BSA (mg)	immobilized BSA (mg)	immobilized BSA (mg/g support)
3.0	1.62	0.38	563
4.0	1.45	0.55	820
5.0	1.60	0.40	596
6.0	1.54	0.46	681
7.0	1.50	0.50	745
8.0	1.48	0.52	774

Table 1: Immobilization of BSA at different pH.

The best pH for BSA immobilization is pH 4.0, which is near the pI value of BSA. It is well-known that immobilization by aldehyde group is an acid-catalyzed nucleophilic addition. At high pH value, carbonyl group is not going to combine with hydrogen ion to form the transition phase. At low pH, it is hard for the amino group

on enzyme surface to provide electron during the second sub-step of the reaction. The result of the competition is an optimized pH value for immobilization, and in this particular immobilization condition, the best pH value is at pH 4.0.

Compared with traditional support, the immobilization capacity for nanosized particles is quite significant. The highest immobilization was at 820 mg/g support, which is nearly ten times higher than micrometric support. Certain biocatalysis limited by enzyme loading will be solved by using magnetic nanoparticles as enzyme support.

## 4 CONCLUSIONS

Spherical magnetic nanoparticles were successfully synthesized and characterized. The mean size of the particle is 100nm with superparamagnetic property. After functionalization to aldehyde group, these particles were further used for immobilization of BSA. Different pH was used for optimization of the immobilization condition. At pH 4.0, the immobilization capacity is 820mg/g support, which is much higher than the normal micrometric support.

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