Fe₃O₄@PDA Immobilized glycerine dehydrogenase

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Abstract. Synthesis of surface modified/magnetic nanoparticles has become a vital research area of material science. In the present work, iron oxide (Fe₃O₄) nanoparticles prepared by solvo-thermal method were functionalized by polydopamine, which was as a facile nanoplatform for immobilizing glycerine dehydrogenase (GDH). The obtained nanocatalyst enabled efficient glycerol dehydrogenation reaction. The results show that GDH immobilized on polydopamine coated iron oxide (Fe₃O₄@PDA@GDH) revealed better adoptability towards higher levels of pH comparative. Moreover, this biocatalyst can be easily recovered from the reaction system without centrifugation or filtration.

1. Introduction

Owing to the properties of nano-materials (high specific area), and super-paramagnetism (easily separated, easy separation from the reaction medium in the presence of an external magnetic field), magnetic nanoparticles are considered as one of the ideal candidates for enzyme immobilization. [1] However, during the interaction of proteins/cells enzymes with naked the magnetic nanoparticles, the rapid agglomeration of magnetic nanoparticles due to high surface area and the lack of surface functional groups in these materials (which lead to the less supporting site) become the raising serious issues. Consequently, surface modification of the magnetic nanoparticles through a self-assembly process has gained increasing attention recently.

Dopamine (DA) can self-polymerize in alkaline aqueous solution with air to form an adherent polydopamine (PDA), which can be easily coated on various substrates, with controllable film thickness and durable stability. [2-4]

In this work, magnetic nanoparticles with uniform particle size and good appearance composite polydopamine (PDA) has been prepared for immobilization of GDH. Firstly, Magnetic nanoparticles with well-defined and uniform were prepared by changing a series of external conditions. Then DA was chosen as the functional monomer to prepare Fe₃O₄@PDA composite. Finally, Fe₃O₄@PDA was used as the carrier for immobilization of GDH. The GDH activity was significantly enhanced and the immobilized GDH shows high pH stabilities.
2. Experimental

2.1. Materials
Beta-Nicotinamide adenine dinucleotide hydrate (NAD$^+$), Glycerol dehydrogenase (GDH, from Cellulomonas sp., 50-125 units/mg protein) were purchased from Sigma-Aldrich. Iron(III) chloride hexahydrate was purchased from Macklin, Sodium acetate trihydrate, Sodium acetate, Trisodium citrate dihydrate, ethylene glycol, ethanol, were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China), High-purity water with a resistivity of 15.0 MΩ was obtained from the Millipore Milli-Q purification system.

2.1.1. Preparation of Fe$_3$O$_4$ nanoparticles. 1.3 g FeCl$_3$·6H$_2$O and 0.5 g sodium citrate and 19mM different alkali sources were mixed to 40 mL ethylene glycol. After vigorous stirring at different temperatures for 1h, the solution was transferred into a 100 mL Teflon-lined stainless-steel autoclave. The autoclave was placed in an oven, and heated to 200 °C for 10 h. After cooling to room temperature, the resulting product was washed with ethanol and deionized water, and then dried in a vacuum oven at 50 °C for 24 h. [5]

2.1.2. Preparation of Fe$_3$O$_4$@PDA nanoparticles. Fe$_3$O$_4$@PDA was prepared via solution of dopamine-hydrochloride added to the aqueous dispersion of Fe$_3$O$_4$ for 4h. In detail, 100 mg of Fe$_3$O$_4$ was added to 40 mL Tris-HCl solution containing 2mg/ml DA was added and the pH of mixture solution was adjusted by the 1M NaOH solution (pH=8.5). The solution was centrifuged under 8000 rpm for 10 min and then the polydopamine coated Fe$_3$O$_4$ (PDA-Fe$_3$O$_4$) was obtained.

2.2. Characterization
The microstructures of as-prepared samples was observed through scanning electron microscopy (transmission electron microscopy (TEM; Tecnai G2 F20 with accelerating voltage of 200 kV). Fourier transform infrared spectra (FTIR) of the samples were obtained from FTIR Spectrometer (FTIR; Bruker Tensor 27). The X-ray diffraction (XRD) analysis was conducted on an XRD-6100X diffractometer (Shimadzu, Japan) with a copper K$\alpha$-radiation source at a scan rate of 5° per min ranging from 20° to 80°.

2.2.1. Enzyme activity. GDH was immobilized by following adsorption process: the PDA modified Fe$_3$O$_4$-nanoparticles were dispersed in a enzyme solution (0.001 mg/mL), which prepared by dissolving a certain amount of GDH in Tris-HCl buffer solution (50 mM) with pH=9. The enzyme activity of immobilized GDH was tested by monitoring the catalytic conversion of glycerol. Briefly, substrate solution was obtained by dissolving glycerol (substrate) as well as NAD$^+$ (cofactor) in Tris-HCl buffer solution (50mM, pH 7.0), and their concentration were 1mM and 1 M, respectively. Subsequently, immobilized GDH (both containing 0.01 mg of enzyme) was added into 10 mL substrate solution and stirring for 30min, and then the change of NAD$^+$ concentration was recorded by measuring the absorbance of NADH at 340 nm. The reaction degree was defined by eq 1:

$$\text{Reaction degree (\%)} = \frac{c}{c_{\text{max}}} \times 100\%$$

Where C (mg/mL) was the concentration of NADH.

3. Results and Discussion
Transmission electron microscopy (TEM) was used to characterize the size and morphology of the Fe$_3$O$_4$ NPs by different alkali sources and different precursor synthesis temperature. During the synthesis of Fe$_3$O$_4$ nanoparticles, sodium acetate with different water content were used as alkali source. The TEM image of Fe$_3$O$_4$ prepared by sodium acetate trihydrate showed that the morphology was irregular and the particle size was heterogeneous. (Figure 1a). [5] The TEM image in Figure 1b showed that the average size of Fe$_3$O$_4$ prepared by sodium acetate was about 200 nm, and the surface of the particles
was relatively smooth. During the preparation of Fe$_3$O$_4$, the micro water content in the system had an important influence on the morphology and particle size of Fe$_3$O$_4$. The synthesis temperature of the precursor was also affect the morphology and particle size of Fe$_3$O$_4$. The corresponding TEM image (Figure 1c, d) confirmed that effect of morphology and particle size of Fe$_3$O$_4$ with different temperature of precursor solution.

![Figure 1. TEM images of the formation of Fe$_3$O$_4$ nanoparticle: Alkali source: (a) Sodium acetate trihydrate; (b) Sodium acetate; the precursor synthesis temperature: (c) 25 °C; (d) 80 °C.](image)

![Figure 2. (a) XRD diffraction patterns of the Fe$_3$O$_4$; (b) FTIR spectrum of the Fe$_3$O$_4$@PDA; (c) Room-temperature magnetization hysteresis loops of Fe$_3$O$_4$ and Fe$_3$O$_4$@PDA.](image)
The phase composition of Fe₃O₄ NPs nanocomposites was confirmed by powder XRD over the range 20° ≤ 2θ ≤ 80° (Figure 2a). As shown in Figure 2a, all the diffraction peaks of the as-prepared Fe₃O₄ NPs could be assigned to face-centered-cubic phase Fe₃O₄ according to the standard XRD pattern (JCPDS 88–0866). The broad peaks at 2θ = 30.1°, 35.5°, 43.1°, 57.0° and 62.6° corresponded to the crystal planes indices of (220), (311), (400), (511) and (440), respectively. [6] The Fe₃O₄@PDA sample was characterized by FTIR, and the corresponding spectra was shown in Figure 2a. The characteristic peaks at 3432 cm⁻¹ was corresponded to the −OH group in the spectra of PDA, respectively. The band at 582 cm⁻¹ (Fe-O) was assigned to Fe₃O₄ nanoparticles. After a PDA modification, the FT-IR spectrum of Fe₃O₄@PDA exhibited distinct peaks at 1617, 1508 cm⁻¹, which were ascribed to C=C stretching vibrations, respectively (Figure 2b). To investigate the composition-dependent magnetic properties of the Fe₃O₄ and Fe₃O₄@PDA, magnetic hysteresis loops were measured and the relative results were showed as Figure 2c.⁷ The M–H curves displayed a similar S-type shape and were saturated under an applied magnetic field of 20 kOe (Figure 4). After PDA coating, saturation magnetization (Ms) exhibited a decrease, because the core-shell NRs were composed of ferromagnetic Fe₃O₄ cores and diamagnetic MPN shells. Although the Ms value of Fe₃O₄@PDA were smaller than the naked Fe₃O₄, the magnetic sensitivity was enough for separating the nanocarriers from reaction systems (Figure 4).

Figure 3. pH stability (a) and thermal stability (b) of Immobilized enzyme

Figure 3(a) shows the relative activity for free and immobilized GDH with the variation of pH values. The optimal pH value remained unchanged (pH 8.0) after immobilization in the Fe₃O₄@PDA nanocomposite. The immobilized GDH keeps higher relative activity than its free form under extreme alkaline conditions till pH 10.0 (95% vs. 46%). It was known that enzyme usually lost activity at high temperature. Thus, activity of GDH was studied at pH 8.0 in the temperature range 30-70°C. As seen in Figure 3(b), high temperature seemed have more influence in free enzyme deactivation and their relative activity linearly decreased to 0 while the temperature increased from 30°C to 70°C. In the same process of temperature increasing, immobilized GDH showed lightly higher temperature tolerance.

4. Conclusion
In summary, We have prepared magnetic nanoparticles with uniform particle size and good appearance through a series of control conditions. And a self-assembly process of PDA could successfully occur onto the surfaces of Fe₃O₄ NPs. The GDH immobilized on Fe₃O₄-PDA showed the highest pH stability. The present work not only provided a series of control conditions for preparing magnetic nanoparticles, but also demonstrated that PDA coating was a facile and universal method for enzyme immobilization.
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