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Design and construction of magnetic nanoparticles incorporated with a chitosan and poly (vinyl) alcohol cryogel and its application for immobilization of horseradish peroxidase

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Abstract

Magnetic nanoparticles were synthesized by simple co-precipitation method in aqueous medium. The structural characteristics of the powders were studied by XRD and the sizes of nanoparticles were measured with dynamic light scattering. The prepared magnetic nanoparticles were about 43 nm in diameter and were then subsequently incorporated into a chitosan and poly (vinyl alcohol) cryogel and applied to immobilize the horseradish peroxidase through covalent binding method (MNP-CS-PVA-HRP cryogel microbar). The MNP-CS-PVA- HRP cryogel microbar was applied for the detection of hydrogen peroxide. The horseradish peroxidase can catalytically oxidize the substrate of hydrogen peroxide and *o*-dianisidine, generating blown colour that are proportional to the concentrations of hydrogen peroxide. The results showed that the absorption values at 430 nm increased with the hydrogen peroxide concentrations ranging from 10-1,000 mM. The immobilized horseradish peroxidase on magnetic nanoparticles retained enzyme activity up to the 10^{th} reusable. The proposed approach confirmed that the magnetic nanoparticles not only possessed enzyme activity but also showed potential application in varieties of simple, robust, and cost-effective analytical methods in the future.

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Keywords: Magnetic nanoparticles; Horseradish peroxidase; Hydrogen peroxide; Poly (vinyl) alcohol cryogel

1. Introduction

Over the past few decades, nanoparticles have much interested due to their own advantages such as high surface area, excellent in chemical, physical and biological properties [1-2]. A variety of nanoparticles with different materials such as gold [3], silver [4], titanium oxide [5] and magnetic nanoparticles [6] have been applied in many

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scientific fields. Among the various nanomaterials, magnetic nanoparticles have been rising as a significant useful material for variety applications. Firstly, magnetic nanoparticles have a very large surface-to-volume and good biocompatibility [2]. Moreover, the magnetic nanoparticles are especially used for concentration, separation, purification and identification of interested analyst [7-8]. The magnetic nanoparticles were synthesized in several forms mostly includes Fe_3O_4 (magnetite), α-Fe_2O_3 (hematite), γ-Fe_2O_3 (maghemite), FeO (wustite), ε-Fe_2O_3 and β-Fe_2O_3, among which magnetite and maghemite are popular candidates since its biocompatibility have already applications in biotechnologies [9]. Magnetic nanoparticles were widely applied for immobilization of enzyme [10], immunoassay [11], bioseparation [12] and biosensor [13]. Magnetic nanoparticles have a wide promise to immobilize enzyme on its surface not only to perform better than conventional bulk supports used in enzyme immobilization but also faster separation by external magnetic field which resulting in immobilized enzyme could be reused. Various attempts have been made to immobilize enzyme on magnetic nanoparticles with different methods including using layer-by-layer [14], electrostatic force [15] and covalent binding [16]. Recently, Kuo and co-workers have been used the Fe_3O_4-chitosan nanoparticles for the immobilization of lipase via covalent method. The results found that, after twenty repeated uses the immobilized lipase retains over 83% of its original activity [17].

In this work, we synthesized magnetic nanoparticles (MNP) and were incorporated into a chitosan (CS) and poly (vinyl) alcohol (PVA) cryogel (MNP-CS-PVA cryogel microbar). This magnetic-CS-PVA cryogel was applied for immobilization of horseradish peroxidase (MNP-CS-PVA-HRP cryogel microbar) for hydrogen peroxide detection. To our knowledge, this MNP-CS-PVA-HRP cryogel microbar is used for the first time as a support material for enzyme immobilization. As shown in Fig. 1, horseradish peroxidase was covalently immobilized on MNP-CS-PVA cryogel microbar by using gultaraldehyde as a crosslink agent. In the presence of hydrogen peroxide, *o*-dianisidine was oxidized by horseradish peroxidase resulting in the color change to blown color [18]. The absorbance value of the colorful products is proportional to the concentration of the hydrogen peroxide.

2. Experimental

2.1. Chemicals and reagents

All chemicals used in this study were of analytical grade. Iron (II) chloride and iron (III) chloride were purchased from POCH SA, Poland. Chitosan with low molecular weight and 75% deacetylation degree, acetic acid, sodium hydroxide (NaOH), 30% hydrogen peroxide solution, poly (vinyl) alcohol (PVA, 87-90% hydrolyzed), *o*-dianisidine and horseradish peroxidase (HRP; EC 1.11.1.7) were obtained from Sigma-Aldrich. Glutaraldehyde was purchased from Fluka. All solution was prepared with high purity water.

2.2. Instrumentation

The size measurements of magnetic nanoparticles were done with dynamic light scattering (DLS, Delsa nano C Particle Analyzer, USA). XRD study was carried out by using X-ray diffractometer (X'Pert PRO, PANalytical) for structural characterization of magnetic nanoparticles. The absorbance measurements were carried out by using a UV-1601 UV-visible spectrophotometer (SHIMADZU, Japan) with a 10 mm path length quartz cuvette sub-micro (16.40/Q/10 Starna, USA).

2.3. Synthesis of magnetic nanoparticles

The magnetic nanoparticles were prepared by co-precipitating of Fe^{2+} ($FeCl_2 \cdot 4H_2O$) and Fe^{3+} ($FeCl_3 \cdot 6H_2O$) ions in base solution with nitrogen gas as the protective gas. Briefly, the 0.3 mmol ferric and 0.6 mmol ferrous salts (molar ratio 1:2) were dissolved in deionized water and were then mixed under nitrogen gas at 90 ºC. Then 2.5 M NaOH was added into above mixture solution with vigorous stirring for 30 min. The black precipitates were formed and were separated by magnetic decantation. Finally, the precipitates were washed with deionized water to remove excess base and were dried at 80 ºC for 3 h. There resulting magnetic nanoparticles were characterized with dynamic light scattering and X-ray diffractometer.