

## Nanoparticles Effects on Performance of Horseradish Peroxidase Biosensor

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### Abstract

The immobilization of horseradish peroxidase (HRP) enzyme causes protections on catalytic activities in determination of H<sub>2</sub>O<sub>2</sub>. The rate of electron transfer from redox enzyme onto the electrode surface is an important topic in bioelectrochemistry that could improve more the function of HRP biosensor by means of increasing electrode conductivity. Referring to this basis, effect of various nanoparticles are evaluated and compared with each other on the operation promotion in this review and Glassy Carbon Electrode (GCE) is utilized as a main foundation on immobilization of nanoparticles and HRP enzyme. Electrodes have been compared by the rate of current transfer in the optimum voltage data in the presence of Carbon-coated Iron Nanoparticles (CIN), Quantum Dots (QDs), Au and Laponite/Chit nanoparticles. Consequently in the same scan rate, HRP/Au/GCE (28  $\mu$ A) has achieved more current which is about 94% comparing to the base electrode HRP/GCE (1.8  $\mu$ A).

**Keywords:** Biosensor; Horseradish peroxidase; Nanoparticles; Bioelectrochemistry

### Introduction

During last 20 years, biosensors have been applied as useful monitoring devices in the environmental researches owing to the advantages such as minimizing the sample pretreatment, reducing cost and time of analysis, displaying sufficient sensitivity and selectivity [1]. Among various biosensors, recent attention has turned toward the inhibition based enzyme sensor, which utilize enzyme as a bioelectrocatalyst. Horseradish peroxidase (HRP) is an important enzyme, which has been studied for more than one century and can be implemented as a critical molecule model. Hydrogen peroxide analyte has been detected to characterize performance of HRP biosensor because it is not only an important by-product formed from a lot of peroxidase-catalyzed reaction but also a signal transducer molecule in food, clinical, pharmaceutical, industrial, and environmental assays [2]. Considering that the electron transmission rate has an important role in the efficiency of biosensors and these operations are carried out by the electrode, the electrode used in biosensors structure must have a high conductivity for electron transmission from reaction zone to the transducer. In new researches to achieve this goal, the nanoparticles are used to improve the conductivity, and good compatibility of electrode structure. In this paper, to increase the performance of HRP biosensor before immobilization of the enzyme to the working electrode surface, the electrode modified by different nanoparticles. A conventional three electrode system was used throughout the electrochemical experiments with a modified or bare glassy carbon electrode as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. The enzyme is immobilized on polymeric electrode surface by trapped immobilization method. Working electrode has been evaluated by place in the amperometric cycle with reference and auxiliary electrode. The amount of analyte is determined by the results of current rate. The impact of nanoparticles on increasing the electrode conductivity is evaluated by coated electrode with nanoparticles.

### Experiments

#### Preparation of glassy carbon electrode

A glassy carbon electrode (GCE 0.3 mm in diameter) was polished with 1.0, 0.3 and 0.05  $\mu$ m Al<sub>2</sub>O<sub>3</sub> slurry successively followed by rinsing

thoroughly with distilled water until a mirror-like surface was obtained. Then it was washed ultrasonically in nitric acid, absolute ethanol and distilled water for 1-5 min, and allowed to dry at room temperature [3-7].

#### Preparation of enzyme solution

HRP solution was obtained by dissolving 5.0 mg of HRP in 1 ml phosphate buffer solution (PBS) (7.0) [5].

#### Enzyme immobilization

Solution containing HRP and different nanoparticles was dropped onto the surface of a cleaned GC electrode. Nanoparticles include: iron coated with carbon (CIN), QD, AUNPs-SF and laponite. Each solution after all, is put in condition of room temperature or refrigerator for a period of time required to get dry [3-7].

### Result and Discussion

According to figure 1, the difference in current rate between GCE and HRP/GCE is about 70%. This result shows the efficiency reduction of binding HRP to the GCE electrode. The nanoparticles are added to the HRP/GCE electrode to increase reduction.

Figures 2-5 show that the current rate is increased about 74.3%, 40%, 94% and 76% by adding CIN, QDs, AUNPS -SF and Laponite to the HRP/GCE electrode respectfully. In order to compare the effect of nanoparticles on the electrode performance, the QD/HRP/GCE efficiency is increased about 40% to compare with the HRP/GCE. The best performance of biosensor is achieved for the gold nanoparticle with about 94% of efficiency. It comes out that however implementing different nanoparticles in the structure of the electrode can increase its

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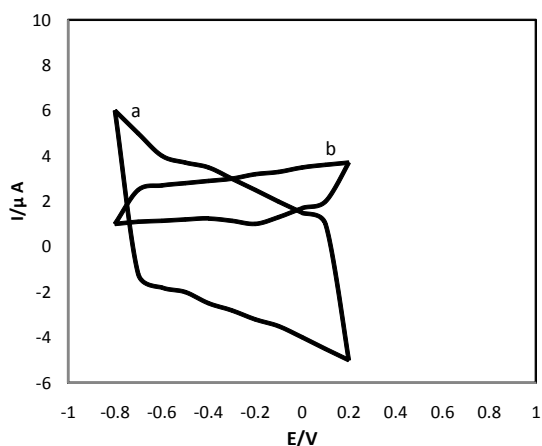


Figure 1: Cyclic voltammograms of: (a) GCE; (b) HRP/GCE.

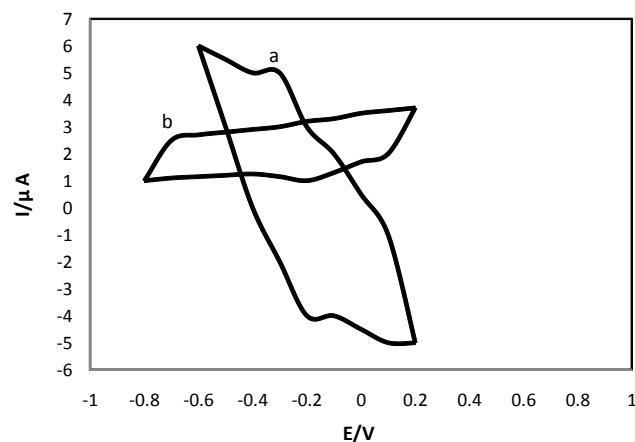


Figure 4: Cyclic voltammograms of: (a) CIN-HRP/PTH/GCE; (b) HRP/GCE.

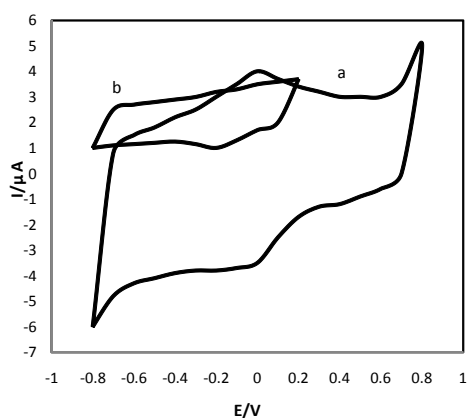


Figure 2: Cyclic voltammograms of: (a) QD+HRP/GCE; (b) HRP/GCE.

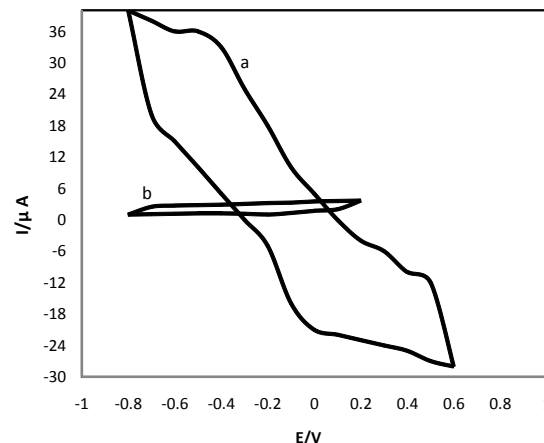


Figure 5: Cyclic voltammograms of: (a) HRP-AUNPS-SF/GCE; (b) HRP/GCE.

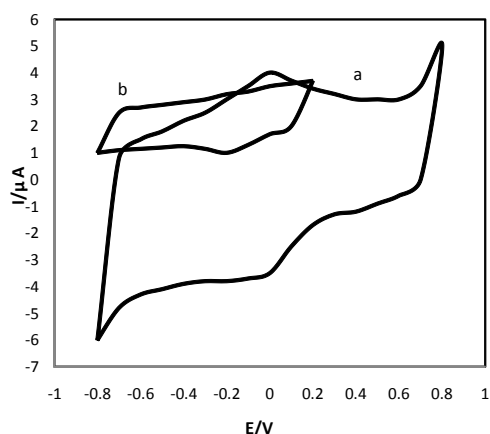


Figure 3: Cyclic voltammograms of: (a) HRP/Laponite/Chit/GCE; (b) HRP/GCE.

conductivity, but nanoparticle's type as well is effective in this improve to such a great extent. To assess these electrodes, the factors such as better conductivity, lower resistance and higher sensitivity in different range of analyte constructions should be measured and the current rate

	HRP/ laponite/ chit/GCE	HRP/Au/ GCE	QD/HRP/ GCE	CIN/HRP/ PTH/GCE	HRP/ GCE	GCE
Optimum current rate	7.5	28	3	7	1.8	6
Optimum voltage	-0.1	-0.4	-0.2	-0.25	-0.2	-0.4
Percentage of difference current rate between electrodes and GCE	20%	78.6%	-50%	14.3%	-60%	

Table 1: Comparison of electrodes performance coated with different nanoparticles.

is necessary to measure these factors for which the results are illustrated in table 1.

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