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Development of a Gold Nanoparticle-Based Electrochemical Biosensor for Detection of Phenolic Compounds

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Abstract: In this study, an enzymatic biosensor was developed with gold nanoparticles and its performance was tested for detection of phenolic compounds. Different combinations of chitosan (Chit), as a support, gold nanoparticle (GNP), and tyrosinase enzyme (T) were coated on glassy carbon working electrode (GCE) to form the enzymatic biosensor. The sensor's components (Chit, GNP and T) were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) while its performance and selectivity to catechol (as a model phenolic compound) were tested by chronoamperometric method. An amplified sensor signal was observed depending on high conductivity of gold nanoparticles. Developed sensor has wide linear range (0.046-50 μM), low detection limit (13.8 nM) and high sensitivity (1.144 A/M). The results reported here indicate that this kind of biosensors is a potential candidate for cheap, fast, and simple detection of phenolic compounds.

Keywords: Electrochemical enzyme biosensor; gold nanoparticle; phenol detection.

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INTRODUCTION

Phenolic compounds have been used in the production process of many important components including pesticides, petrochemical products, and wood protectors (1). These compounds are harmful for environment because of their toxicity (2) threating human health and ecological balance (3). Therefore, true and fast detection of phenolic compounds becomes significant. The methods such as spectrophotometry and chromatography are unpractical due to the fact that they are time consuming, expensive and they require trained personnel (4). Biosensor technology is a promising alternative for detection of phenolic compounds (5, 6).

The working principle of biosensors is based on the measurements of the sensor signal that results from the interaction of the analyte with the electrode surface coated by the biofilm (7). Biological agents which will react with the analyte can be enzyme, nucleic acid, microorganism, tissue, antibody, or cells (8). Enzymatic biosensors depending on their substrate interaction capabilities and biocatalytic properties have been developed by using the enzymes as a biological element such as glucoseoxidase (GOD), horseradish peroxidase (HRP) and glutamate dehydrogenase (GDH) for the detection glucose, heavy metals, and pesticides (9). Besides, tyrosinase enzyme is cheaper and extensively studied for detection of phenolic compounds.

Various polymers have been used as support materials for enzyme immobilization. Among them, chitosan is the natural and biocompatible biopolymer and it has special properties such as high mechanical strength, permeability, antimicrobial, cheap, harmless nature, and good biofilm capability (10, 11). Chitosan produced by deacetylation of chitin from mushroom or insect's skeleton interacts with materials having protein structure because of its ability of protonation in acidic conditions (12).

Due to their high surface area, nanoparticles are widely used for sensor construction in order to achieve sensitive detection of lower analyte concentration with wide linear range. Especially gold nanoparticles increase the surface stability for enzyme immobilization (13). These nanoparticles are favored as a sensor component with chitosan based biosensor due to being ability to form biofilm with excellent electrical properties as a result of interaction with positive charges (14). Therefore, they allow electrochemical sensing by providing electrochemical communication between enzyme and bulk electrode materials without the need of external electron transfer mediators (15).

In this work, an electrochemical enzyme biosensor is developed based on tyrosinase immobilized chitosan-gold nanoparticle films coated on working electrode to detect the catechol as a model phenolic compounds.

MATERIALS AND METHODS

Chemicals

Tyrosinase from mushroom (T3824, 25KU/4.3mg), chitosan (Chit), gold nanoparticles (GNP, 20 nm, in 0.1 mM phosphate buffer solutions suspension), catechol (Cat), potassium nitrate (KNO_3), and potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6].3H_2O$) were purchased from Sigma Aldrich. Hydrochloric acid (HCl, 37%), disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) and sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$) were purchased from Merck. All solutions were prepared with ultra pure water (18.2 MΩ).

Apparatus

All electrochemical measurements (cyclic voltammetry, electrochemical impedance spectroscopy, and chronoamperometry) were conducted by using triple electrode system (reference electrode: Ag/AgCl, counter electrode: Pt wire and working electrode: 5 mm glassy carbon electrode).

Experimental Procedure

A 50 mM pH: 6.5 phosphate buffer solution (PBS) was prepared with $Na_2HPO_4.2H_2O$ and $NaH_2PO_4.H_2O$. Tyrosinase (T) stock solution (4.3 mg/mL) and 2 mM Cat solution were prepared with 50 mM pH: 6.5 PBS. Chitosan powder was dissolved in 0.1 M HCl while GNP solution was first exposed to ultrasonication for 4 hours and an additional 15 minutes of sonication was conducted before each use. 0.5 mM $K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 solution was prepared with 18 MΩ cm^{-1} ultra-pure water for CV and EIS characterizations.

The surface of 5 mm GCE was cleaned by ultrasonication, polishing with 0.05 μm alumina powder and then washing with ethanol and water to achieve a better biofilm coating. 10 μL of sensor components obtained according to Table 1 were dropped on 5 mm cleaned GCE and then dried in vacuum oven (40 cm Hg, 25 °C) about an hour. Enzyme containing electrodes (Chit-T, Chit-GNP-T) was washed in 10 mL pH: 6.5 PBS solution (200 RPM) for 30 minutes to remove non-immobilized enzymes on electrode surface.

Table 1: Combining ratio of biocomposite films.

Sensor component	Mixing with volume ratio respectively
Chit	From stock solution
Chit-GNP	1/1
Chit-T	1/4
Chit-GNP-T	1/1/4

Impedance measurements were performed in the frequency range of 10^5 - 0.1 Hz with 0,005 V amplitude while CV measurements were conducted both in 0.5 mM $K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 solution and pH 6.5, 0.1 mM Cat containing PBS solution in ±0.6 V potential range and at 0,01 V/s scan rate. Optimum working potential of developed enzyme electrode (Chit-GNP-T) was determined by potential scanning in the range of ±1 V both in the absence and presence of 0.1 mM Cat containing PBS solution. Reusability of the biosensor was tested by 30 successive determinations in 10 µM Cat solution. Analytic performance of the biosensor was determined by chronoamperometric method in the range of 0.2-145 µM Cat concentration. Lower detection limit (LOD) was also calculated by using the equation:

$$LOD = \frac{(3.S_{y/x})}{a} \quad \text{(Eq. 1)}$$

Where *a* is the sensitivity (linear part of calibration curve for Cat) of the method and $S_{y/x}$ is the standard deviation of the fit.

RESULTS AND DISCUSSION

Interface properties of biocomposite films were determined by EIS characterization. Semicircle diameters shown in Figure 1 indicate the electron transfer resistance which controls the electron transfer kinetic on electrode surface. Due to non-conductive properties of Chit and T, increase in semicircle diameter was observed after coating the surface of GCE with Chit and Chit-GNP-T. On the other hand, Nyquist curve diameter decreased with the addition of conductive GNP to the Chit structure (Chit-GNP). Electron transfer resistances were evaluated by fitting the Nyquist curves to an equivalent circuit model in order to clarify the interface mechanism.

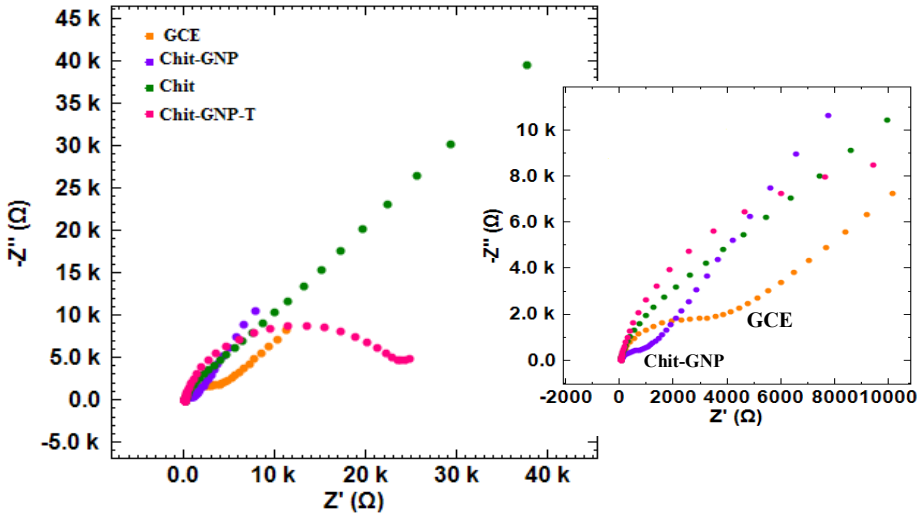


Figure 1. Nyquist diagram of GCE, Chit, Chit-GNP and Chit-GNP-T coated electrode in 0.5 mM $K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 solution. Frequency range: 10^5-0.1 Hz, amplitude: 0,005 V.

In the equivalent circuit model shown in Figure 2, R_s refers to solution resistance, R_{ct} refers to electron transfer resistance, $Y0_1$ and $Y0_2$ refers to constant phase elements.

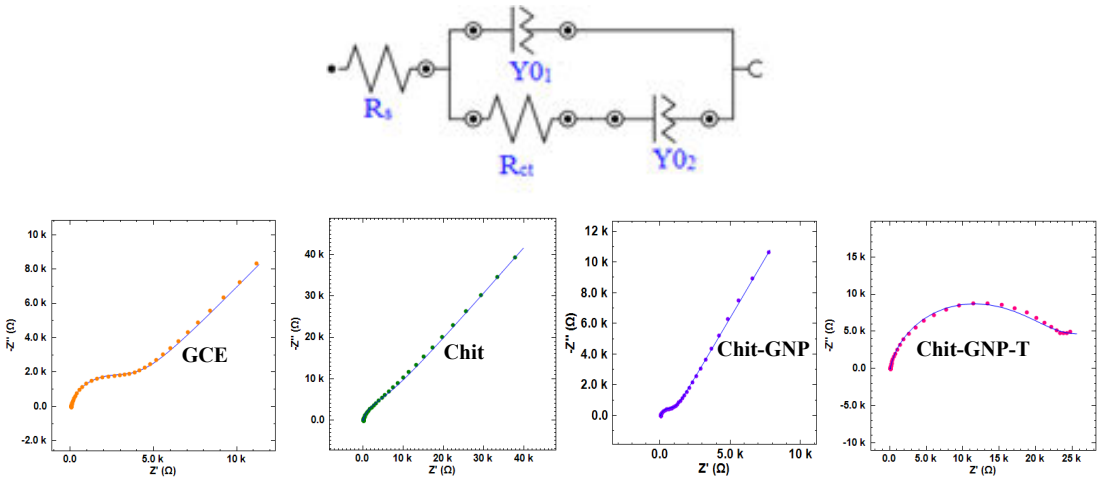


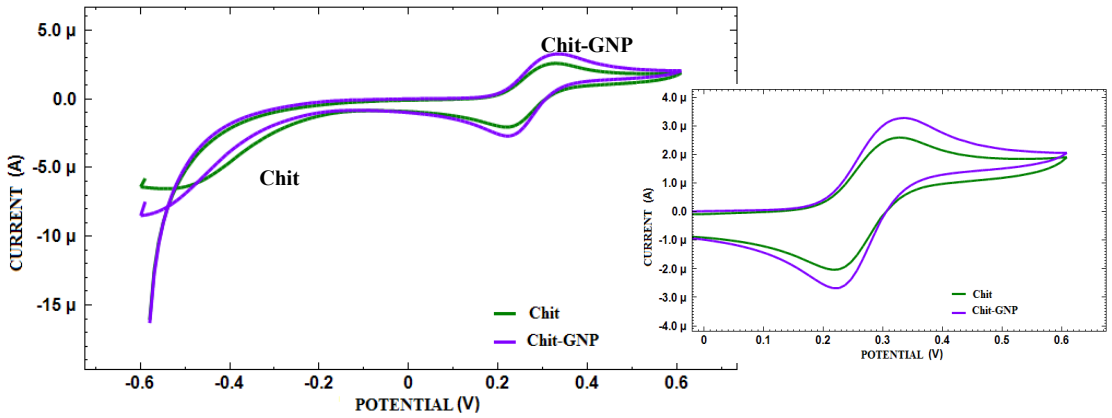
Figure 2. Nyquist diagram of GCE, Chit, Chit-GNP and Chit-GNP-T coated electrode fitted to equivalent circuit model.

The electron transfer resistance (R_{ct}) was estimated as 3.72 kΩ, 6.84 kΩ and 15.6 kΩ for the GCE, Chit and Chit-GNP-T respectively as represented in Table 2. It indicates that Chit and Chit-GNP-T obstructed the electron transfer of $[Fe(CN)_6]^{4-}$ which causes an increase in the electrode impedance. In addition, the obvious increase in R_{ct} for Chit-GNP-T point out the successful immobilization of tyrosinase. The decrease in R_{ct} value for Chit-GNP (1.06 kΩ) with respect to Chit (6.84 kΩ) indicated that GNP accelerate the electron transfer of the electrochemical probe.

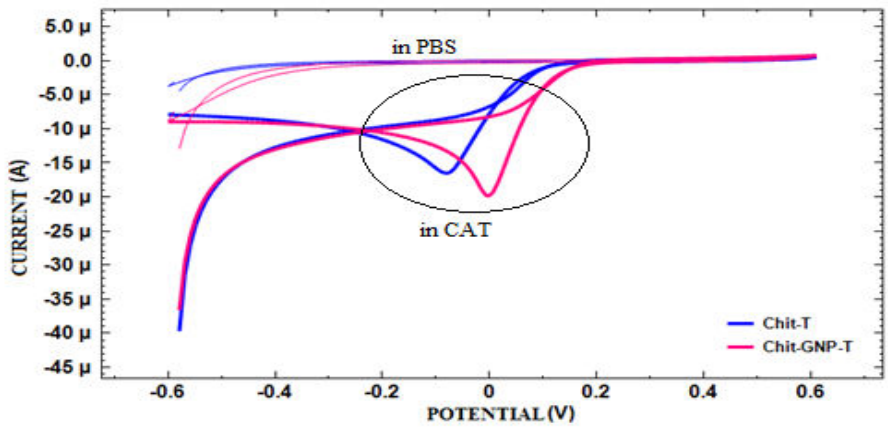
Table 2. Electrical paremeters of GCE, Chit, Chit-GNP and Chit-GNP-T coated electrodes.

Electrode	R_s (Ω)	R_{ct} (kΩ)	Y01 (μMho)	Y02 (μMho)
GCE	14.6	3.72	3.45	109
Chit	14.4	6.84	2.72	21.9
Chit-GNP	13.5	1.06	4.62	101
Chit-GNP-T	14.8	15.6	2.94	98.0

CV measurement was also performed in 0.5 mM $K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 solution to demonstrate the enhancement of GNP used for biosensor design. An increase in the redox peak was observed (Figure 3a) in Chit-GNP biocomposite film due to rapid electron transfer depending on conductive properties of GNP parallel to EIS result. Figure 3b depicts the cyclic voltammograms of Chit-T and Chit-GNP-T coated electrodes in the absence and presence of 0.1 mM Cat containing PBS solution. There was no redox peak in the absence of Cat while distinct reduction peak was observed in its presence both for Chit-T and Chit-GNP-T coated electrodes.



(a)



(b)

Figure 3. Cyclic voltammetry of Chit and Chit-GNP coated electrodes. Potential range: ±0.6 V, scan rate: 0.01 V/s. (a) in 0.5 mM $K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 solution. (b) in the absence and presence of 0.1 mM Cat containing PBS solution.

The observed reduction peak was attributed to the reduction of o-quinone on the electrode surface shown in Figure 4. Comparison of the reduction peak currents at Chit-T and Chit-GNP-T clearly demonstrates that GNPs result in a dramatic enhancement of the electrochemical signal (Figure 3b). Therefore the most suitable sensor components were chosen as Chit-GNP-T.

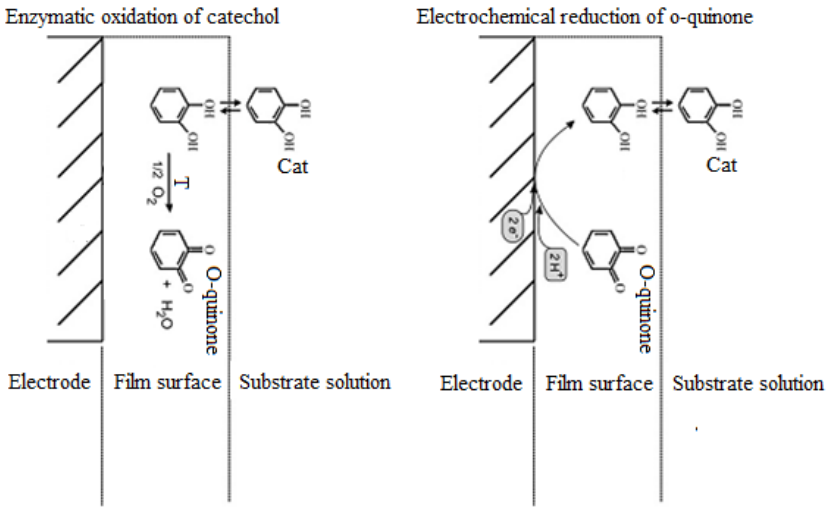


Figure 4. Enzymatic oxidation of catechol and electrochemical reduction of o-quinone.

In this work, optimal working potential was determined to detect the Cat chosen as the phenolic compound by using amperometric method. A higher signal difference was obtained at -0.6 V potential value which was used for further amperometric measurements as seen in Figure 5.

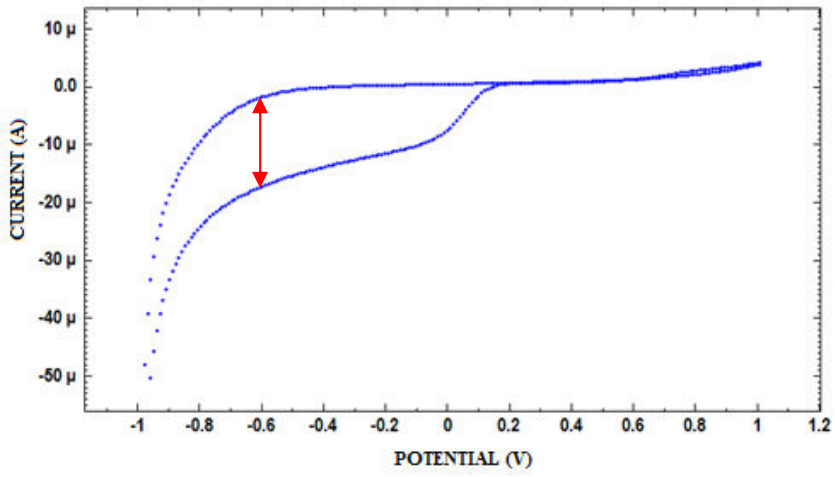


Figure 5. The effect of working potential on amperometric response of developed sensor (Chit-GNP-T) in the absence and presence of 0.1 mM Cat containing PBS solutions.

The phenolic compound "Cat" was detected by developed sensor (Chit-GNP-T) in the concentration range of 0.2-145 µM and calibration curve (Figure 6) was obtained from typical time-current plot (amperometric responses). Kinetic parameters (K_m, I_m) were also calculated by fitting the experimental data to Michaelis-Menten model equation.

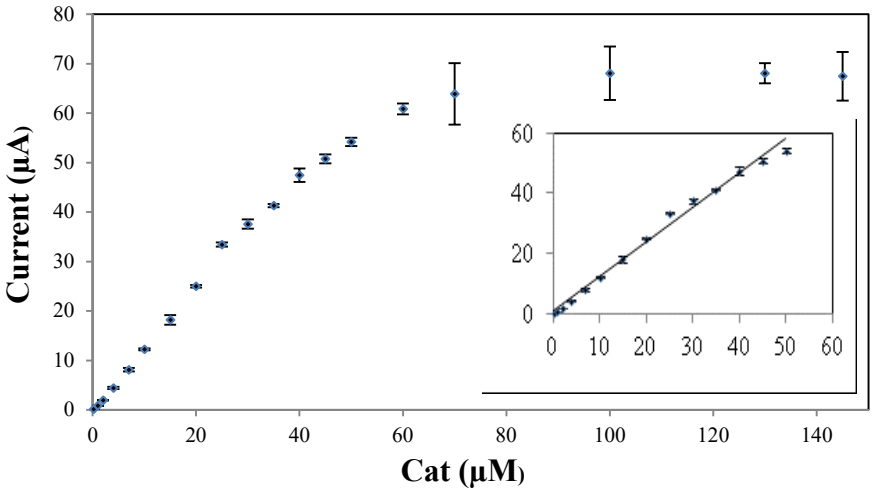


Figure 6. Calibration curve for Cat obtained Chit-GNP-T biosensor.

Analytical performance of developed biosensor including linear range, correlation coefficient, detection limit and sensitivity are summarized in Table 3. Chit-GNP-T biosensor has wide linear range with high correlation coefficient. Compared with the literature, Chit-GNP-T biosensor developed in this study has low detection limit (13.8 nM) with highest sensitivity. The low K_m value demonstrated that Chit-GNP-T biosensor exhibited a high affinity to Cat. Relative standard deviation value of 3.1% for ten successive measurements also indicates good repeatability for developed biosensor.

Table 3. Analytical performance of developed biosensor in Cat containing media.

Analytical performance	This work	Wang *et al*.	Han *et al*.	Obrero *et al*.
Linear range (µM)	0.046-50	0.083-70	0.001-20	0.2-10
Correlation coefficient	0.991	0.999	0.997	0.999
Detection limit (nM)	13.8	25	0.3	353
Sensitivity (A/M)	1.144	0.514	0.561	0.055
K_m (µM)	47.7	96.9	5.21	84.4
I_m (µA)	98.3	83.6	-	-

In this work, an electrochemical biosensor enhanced with gold nanoparticles was developed for detection of phenolic compounds. Sufficient amount of enzyme loading was provided by nanobiocomposite film as understood from electrochemical

characterizations. The designed biosensor demonstrated good analytical performance without extra mediators to increase electrode signal, and showed high sensitivity, low detection limit, and good reusability. The design strategy can lead to develop different types of electrochemical enzyme biosensor.

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Türkçe Öz ve Anahtar Kelimeler

Fenolik Bileşiklerin Tayini İçin Altın Nanopartikül Esaslı bir Elektronik Biyosensörün Geliştirilmesi

Melike KIZILKAYA, Ülfet EREN, İlker POLATOĞLU

Öz: Bu çalışmada, altın nanopartikül kullanılarak bir enzimatik biyosensör geliştirilmiş ve fenolik bileşiklerin tayininde kullanılmıştır. Farklı kitosan (Chit), destek olarak, altın nanopartikül (GNP) ve tirozinaz enzimi (T) camsı karbon çalışma elektrodu (GCE) üzerine kaplanmış ve enzimatik biyosensör oluşturulmuştur. Sensörün bilşenleri olan Chit, GNP ve T döngülü voltametri (CV) ve elektrokimyasal impedans spektroskopisi (EIS) ile karakterize edilmiş ve model fenolik bileşik olarak katekole karşı seçimlilik performansı test edilmiştir. Altın nanopartiküllerin yüksek iletkenliği nedeniyle artmış bir sensör sinyali gözlenmiştir. Geliştirilen sensörün geniş bir lineer aralığı (0,046 – 50 µM), düşük tayin sınırı (13,8 nM) ve yüksek hassasiyet (1,144 A/M) içerdiği bulunmuştur. Burada bildirilen sonuçlara göre bu türden biyosensörler fenolik bileşiklerin ucuz, hızlı ve basit bir şekilde tayinine olanak tanıyan potansiyel adaylardır.

Anahtar Kelimeler: Elektrokimyasal enzim biyosensörü; altın nanopartikül; fenol tayini.

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