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Amperometric Phenol Sensors Employing Conducting Polymer Microtubule Structure

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An amperometric sensor for the determination of phenolic compounds in waste water was developed adopting microtubule structure of conducting polymer. The tubule structure used for enzyme immobilization was manufactured by the electrochemical polymerization of EDOT into a polycarbonate template membrane. The microtubule worked as a container retaining enzymes as well as a current collector for the electrochemical reaction of the species inside. Enzyme species, for example tyrosinase stored inside the tubule converts the phenolic substrates into quinones. The quinone product was an electrochemically detectable product, which was detected at $-100\,mV$ vs. $Ag/AgCl$ by the tubule electrode. The calibration curve showed linearity in the range of $0{\sim}6\,\mu M$ of phenols. The Sensitivities of the biosensor for catechol were $3.1\,\mu A/\mu M \cdot cm^2$.

Keywords Conducting polymer; enzyme; microtubule; phenol sensor; tyrosinase

1. Introduction

Phenolic compounds are widespread in nature, and play a crucial role in living organisms. They are widely used in industries including wood processing and pesticide production. The degradation products of nonionic surfactants which are used both in household products, i.e., textile, food, and in industries of paint and varnish, are another source line of phenol derivatives. Because of this, phenols occurring in waste water and soil pollute the environment seriously. Many of these compounds are highly toxic, and their determination in low concentrations has been an important issue. Various methods for determining these compounds are well known [1,2]. New procedures for determining phenols with biosensors have been described. Many of matrices such as carbon paste, graphite-epoxy composite have been employed for the incorporation of the tyrosinase enzyme on the electrode surface [3–6]. Sol-gel chemistry offers new and interesting possibilities in the fields of chemical sensors and biosensors [7–11]. Tyrosinase-based biosensors for the determination of phenolic compounds in the organic phase have been reported extensively [12–15].

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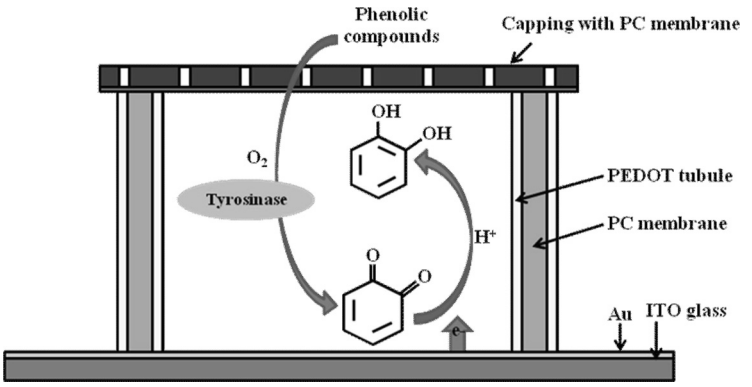

Scheme 1. Schematic diagram of the sensor reaction in capped PEDOT tubule.

The sensitivity of analysis and the stability of an enzyme electrode can be improved using new matrices for enzyme immobilization. However, conducting polymer matrix for the reduction based amperometric sensor was hardly found so for. Because conducting polymer itself gets non conductive state by the externally applied reducing potential.

In this work, we tried to show conducting polymer microtubules were utilized to fabricate a phenol sensor by immobilizing enzyme, tyrosinase, into them and playing its roles even under the reducing potential condition. To prevent tyrosinase from escaping out of the containers, the tubule openings were sealed with PEDOT/PSS composite. The film formation and hydrophilic properties of PEDOT/PSS dispersion allows that the polymer can be used to confine bioactive materials such as enzymes in the hollow space of the tubule [16]. The schematic diagram of phenol sensing in this system is shown in Scheme 1. This system can modulate the quantity of enzyme just by loading different concentration of enzyme solution before capping the tubule container. Enzymes in the tubule are free because the capping procedure took place at the outer surface of PEDOT/PSS tubule. This will help reducing the degradation of the enzyme activity during the immobilization process. We will discuss experimental variables affecting the electrochemical response of the biosensor such as operational potential and others.

2. Experimental

EDOT (3,4-ethylenedioxythiophene) was used as received from Sigma Aldrich. PVA (polyvinyl alcohol) was donated by OCI (Korea). Aqueous dispersion of PEDOT/PSS (Baytron P 4083) was used as purchased. Polycarbonate membrane filters (Isopore) having 0.05, 1.2 μm pore diameter were purchased from Millipore and used as templates. The membrane of 1.2 μm pore was coated with gold one side and used for the microtubule template. The other was used for capping the open mouth of the tubule. Tyrosinase (5370 unit/mg solid) were obtained from Sigma-Aldrich. Phosphate buffered saline (PBS) solution consisted of 0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, and 0.15 M NaCl and was adjusted to pH 7 with 3 M NaOH. Other chemicals used in this study were mostly of ACS grade. The conducting ITO glass (Samsung Corning, Korea) was utilized as a supporting

electrode. Spin coating was performed with an EC101DT spin-coater (Headway Research, Inc., USA) equipped with a vacuum pump. Au layer was coated on one side of 1.2 μm membranes by thermal evaporation (Korea Vacuum). Electrochemical treatments and measurements were performed using a BAS 100B (BAS, USA) electrochemical work station and electrochemical potentials in this study were referred to a Ag/AgCl (Sat'd KCl) reference electrode.

The fabrication of hollow microtubule electrodes has been described in detail elsewhere [17,18]. The procedure for making enzyme-imprisonment version of the electrode is described briefly. Homemade conducting glue (PEDOT/PSS + PVA composite) was spin coated onto an ITO glass with spinning rate of 6000 rpm. Then a piece of membrane template was carefully placed on top of it before the glue was dried out completely. The gold coated side of the template was placed to face the bottom ITO. The glue composite has two important roles, firstly gluing the template onto the ITO electrode and secondly behaving itself as an electrode for electropolymerization. The electrochemical polymerization was performed with the template electrode to make conducting polymer tubules into the template pores. Potential cyclic methods was used with the applied potential ranging from 0.3 to 1.2 V at a rate of 50 mV/s. Tyrosinase solution was applied to the open mouths of the tubule array. Before drying, this microtubule array was covered with a piece of 0.05 μm porous membrane on the bottom side of which was coated with the conducting glue. When not in use, the electrode was stored in a refrigerator at 4°C.

3. Results and Discussions

When PEDOT was deposited as a microtubule form, the monomer oxidation current rapidly increased at a certain potential range during the potential cycles [19,20]. The microtubules need to be wet to work and not to be dissolved by water because the amperometric detection is to the performed in aqueous environment. Therefore, the electrochemical polymerization was conducted in 30% water containing acetonitrile electrolytic polymerization solution. Figure 1 shows the polymerization cyclic voltammogram of EDOT. During this procedure, PEDOT tubules were formed in the membrane cavity. The formation of conducting polymer tubule was realized by the employment of the conducting glue which offers electrical contact between base ITO electrode and electrolytic solution. The increases in current at the potential greater than 1.0 V is due to the oxidation of monomer EDOT and the oxidized EDOT radical species coupled to form a polymer chain. By the reversing of the applied potential, polymerization current is decreased and reduction current is appeared at the potential less than 1.0 V. There is no need to apply the potential too low (<0.3 V) to save time. By switching the scan direction at 0.3 V, the increase in polymer oxidation current is appeared. As the number of cycle increases the responding current also increases. This means the tubule is gradually being formed. After 15 potential cycles, the electrode was taken out of the polymerization electrolyte, and cleaned with distilled water and keep in dry. Figure 2 shows the SEM image of tubule structure after removing template. Then, the tubule was filled with enzyme solution by placing the solution on top of the tubules and capping process was completed by putting a nano-porous membrane (0.05 μm diameters) coated with the glue composite on one side only onto the mouth opening of the microtubule formed in the template.

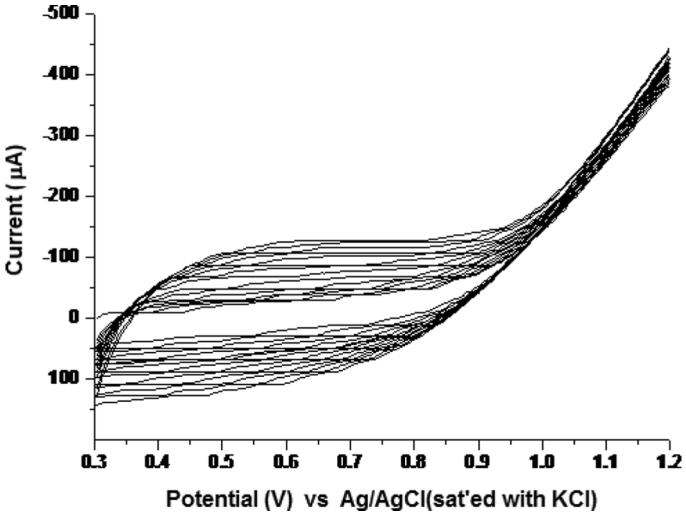

Figure 1. 13 polymerization cyclic voltammograms for preparing conducting polymer tubules into PC membrane (pore size = 1.2 μm). Polymerization solution was a 30% acetonitril solution of 0.1 M EDOT and 0.1 M $LiClO_4$.

To investigate whether these electrodes respond to the substrate, catechol solution was made with buffer solution for the amperometric detection. Figure 3 shows amperometric current responses with different applied potentials after adding catechol solution. Concentration of the test solution was varied from 0.1 μM to 10 μM range and the applied potentials were −0.2, −0.1, and 0.0 V. Both potentials of 0 V and −0.1 V show quite similar responding current scales but the one due to the potential of −0.2 V shows about half of the others. This is quite different result from what we expected. One can easily expect that the potential of −0.2 V can reduce

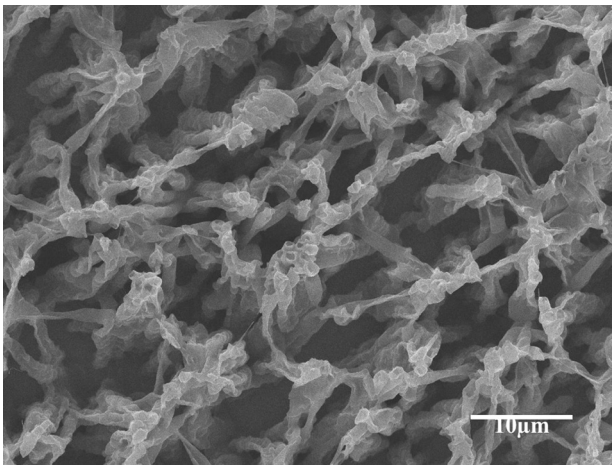

Figure 2. SEM image of the tubule structure formed by electrochemical polymerization of EDOT (after removing the template PC membrane).

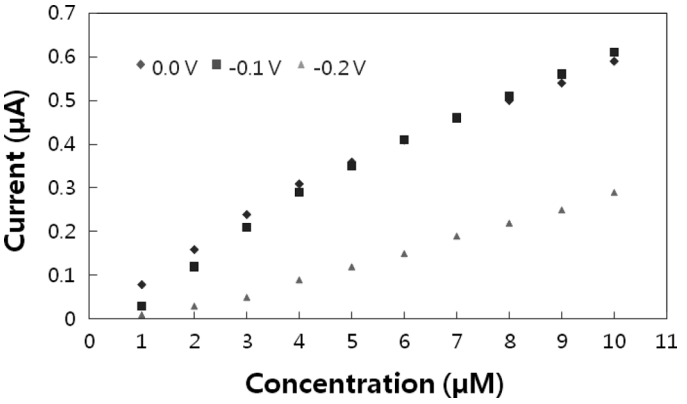

Figure 3. Amperometric current responses of the biosensor to a successive addition of 1 μM catechol under several applied potential. Applied potentials for amperometric detection were 0.0 V, −0.1 V, and −0.2 V.

quinine species produced by the enzyme much faster than the potential of −0.1 and 0 V. So the responding current given by −0.2 V should be greater than those produced by other potentials, but the response appeared in the figure is about half of others. The reason for this is based on the decrease of conductivity of PEDOT. Generally the reducing potential makes the conducting polymer less conductive. PEDOT tubule under −0.2 V is less conductive than that of −0.1 and 0.0 V. Therefore, the responding current given by −0.2 V is decreased. For that reason the detection potential of −0.1 V was used in this study. One advantage of immobilizing enzymes in this type of tubules is modulating the quantity of loaded enzyme freely just by changing the concentration of enzyme solution for better activity. The effect of the concentration of enzyme solution to the response current of the enzyme electrode is shown in Figure 4. Each of tubule electrodes was filled with enzyme solution having 4000, 8000, and 10,000 unit/ml respectively. Measured current scale and detection range is increased by increasing amount of enzyme

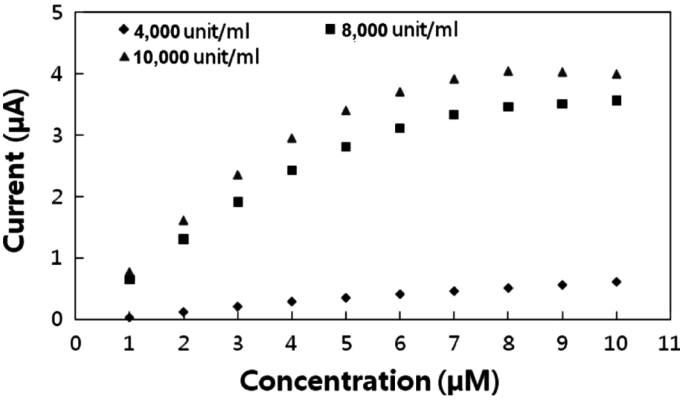

Figure 4. Responses of enzyme electrodes prepared from different concentration of enzyme solution.

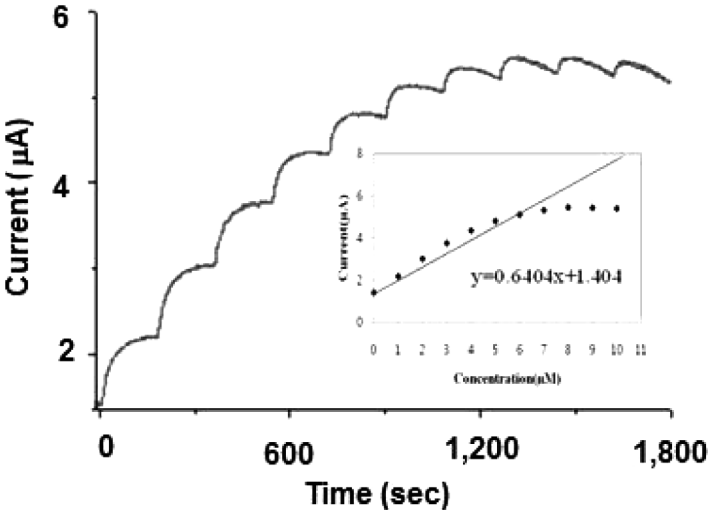

Figure 5. Amperometric responses of a phenol sensor using 10000 unit/ml enzyme to successive addition of 1 μM catechol. The inset is the calibration curve.

loaded in the tubule. The sensor loaded with 4,000 unit/ml solution produces the lowest current response compare to the others. Use of 10,000 unit/ml tyrosinase solution provided the most effective detection. When the amount of enzyme increased, higher than 10,000 unit/ml little change in current was observed (not shown). Figure 5 is the amperometric responses of the electrodes (10,000 unit/ml tyrosinase sensor) in 10 ml of PBS solution. In this experiment the ground current stabilization was carefully achieved before adding substrate catechol. A dose of catechol sample solution was added to the test solution to make the solution concentration 1 μM first. The same amount of catechol solution was added for the next test after 200 sec reaction time. During this period the responding current show rapid increase and stays constant. On the consequent steps the current change shows the same pattern until fifth addition of substrate. After fifth dose the current change shows a little decrease after the quick increase. This may be due to the low concentration of O_2 in the tubule compare to the substrate concentration after eighth dose, this will be assigned in detail later. With the increment of injection concentration, the detection current increases instantly and shows good linearity up to 6 μM. The time required for generating a steady-state current signal was within ~100s. The sensitivity was obtained to be $3.1\,\mu A/\mu M \cdot cm^2$ on average. Published results are shown in Table 1 for comparison. Phenol sensor based on TMOS stock sol-gel solution [21,23] and titania sol [22] produced relatively higher sensitivity than that of present work. But other sensors based on copolymer film [24] and silica sol-gel solution [25] showed lower value in sensitivity. Compare to them, this work shows relatively good sensitivity. However we like to introduce the simple fabrication process for making microtubules which can immobilize various enzyme species in them as a strong point of this work. But response time and long-term stability of the proposed phenol sensor is worse than others. To improve long-term stability, we have to find better permeating materials for capping. Sensitivity enhancement could be done by the use of high poredensity polycarbonate membrane.

Table 1. Comparison of phenol sensors based on different immobilization

Fabrication method	Sensitivity	Reference
Silica sol-gel immobilized amperometric biosensor for the determination of phenolic compounds	$9.1\,\mu A/\mu M \cdot cm^2$	[21]
Titania sol-gel-derived tyrosinase-based amperometric biosensor for determination of phenolic compounds in water samples.	$7.1\,\mu A/\mu M \cdot cm^2$	[22]
Amperometric phenol biosensor based on sol-gel silicate/Nafion composite film	$2.9\,\mu A/\mu M \cdot cm^2$	[23]
Amperometric phenol biosensor based on covalent immobilization of tyrosinase onto an electrochemically prepared novel copolymer poly(N-3-aminopropyl pyrrole-co-pyrrole)film	$5.5 \times 10^{-2}\,\mu A/\mu M \cdot cm^2$	[24]
Silica sol-gel composite film as an encapsulation matrix for the construction of a amperometric tyrosinase-based biosensor	$0.5\,\mu A/\mu M \cdot cm^2$	[25]
This work	$3.1\,\mu A/\mu M \cdot cm^2$	

4. Concluding Remarks

A polymeric microtubule array for hosting tyrosinase enzyme species was fabricated by electrochemical polymerization of EDOT into a polycarbonate membrane template. The cylindrical microstructure has free space in its interior, and that was used to house the tyrosinase. Using the microtubule structure, enzyme tyrosinase was immobilized into the tubule successfully without enzyme modification. After, the mouth of the tubule structure was capped with the PEDOT/PSS glue, phenolic compound determination was performed. Amperometric detection of the enzyme reactive product catechol was performed. By applying reduction potential of -0.1 V, the best current response appears at under the condition of pH 7. The maximum enzyme loading concentration was 10,000 unit/ml. Finally, this biosensor shows linear response to the catechol in $0\sim6\,\mu M$ range with the good sensitivity of $3.1\,\mu A/\mu M \cdot cm^2$. A higher poredensity polycarbonate membrane is needed to improve the sensitivity.

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