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Modified Poly(pyrrole) Film Based Biosensors for Phenol Detection

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***Abstract*—** In order to detect and quantify the phenolic contents of a wastewater with biosensors, two working electrodes based on modified Poly(Pyrrole) films were fabricated. Enzyme horseradish peroxidase was used as biomolecule of the prepared electrodes. Various phenolics were tested at the biosensor. Phenol detection was realized by electrochemical reduction of quinones produced by enzymatic activity. Analytical parameters were calculated and the results were compared with each other.

***Keywords*—**Carbon nanotube, Phenol biosensor, Polypyrrole, Poly(glutaraldehyde).

I. Introduction

PHENOLIC compounds are among the major contaminants in medical, food and environmental matrices [1]. Some techniques such as spectrophotometry and chromatography have been employed for the determination of phenol. However, these methods are usually expensive, time-consuming, and sometimes require sample pretreatment that increases the risk of sample loss and their sensitivity and procedures limit the in-situ applications [2]. Instead of these conventional methods, biosensors could be a cheap and easy alternative measurement method, getting increasing attention in the literature [3], [4]. A biosensor is a self-contained integrated device, consisting of a biological recognition element in direct contact with a transduction element, which converts the biological recognition event into a useable output signal [5]. Owing to their specificity and sensitivity, amperometric enzyme biosensors have been developed for many applications such as electrochemical immunoassays, water pollutants detection and monitoring of biological metabolites [6]. Most amperometric biosensors for the detection of phenolic compounds have been introduced as a mono-enzyme system using tyrosinase, laccase or horseradish peroxidase (HRP) [7]. HRP based biosensors are most sensitive for a great number of phenolic compounds since phenols can be act as electron donors for peroxidase [8]. Recent research activities have focused on the design and construction of modified working electrodes which are most effective to achieve faster enzymatic reaction and electron flow in biosensors.

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In this paper, we discussed measurement performance of two different modified Poly(pyrrole) (PPy) film based phenol biosensors prepared by immobilizing the HRP on gold electrodes via entrapment and chemical bond. Various phenolic compounds were tested at each electrode. Analytical measurement parameters of the biosensors were calculated and compared with each other.

II. Materials and Methods

A. Reagents

HRP (E.C.1.11.1.7) with an activity of 10.000U/vial (according to pyrogallol method performed by the supplier), aqueous solution of hydrogen peroxide (35%), phenol (purity of 99%), glutaraldehyde (25% w/v), lithium chloride, di-potassium hydrogen phosphate and potassium di-hydrogen phosphate were purchased from Merck. Pyrrole and sodium dodecyl sulfate (SDS) and phenol derivatives were obtained from Sigma. Multiwalled carbon nanotubes (CNTs) were obtained from Nanocs. Inc., Newyork, USA.

B. Experimental Setup

All electrochemical experiments were performed by using a CHI 800B Model electrochemical analyzer. Three-electrode system included the gold (Au) working electrode, a Pt wire counter electrode, Ag/AgCl (3 M NaCl) reference electrode and a conventional three-electrode electrochemical cell was obtained from CH Instruments. Amperometric measurements were conducted at a fixed potential of -50 mV. The reaction medium consisted of 100 mM, pH 7 potassium phosphate buffer including, 0.7 mg/mL of lithium chloride as the supporting electrolyte. Three-electrode system was immersed into the reaction medium and, analyzer was started. After reaching a steady-state current, increasing concentrations of phenolic compounds were added to the medium. The amperometric currents were recorded as current-time recordings. The experimental setup was presented in Fig. 1.

C. Fabrication of Working Electrodes

PGA/PPy/HRP/Au working electrode was prepared as follows: polyglutaraldehyde (PGA) solution was prepared by adding 2 mL of 0.1 M NaOH and 2 mL of 25% glutaraldehyde into 10 mL of distilled water. The final solution was stirred at 600 rpm for 30 minutes up to the reaching a final pH of 9-10. The pyrrole polymerization medium was comprised of 100 mM pyrrole and 0.6 mg/mL SDS in 10 mL of PGA solution. The potential was scanned between 0 and +1.2V for electropolymerization of pyrrole. Au electrode was immersed in 25% glutaraldehyde solution and stored at +4°C overnight

after the polymerization step. Then the electrode was immersed in 0.3 mg/mL of HRP solution for 20 hours for the chemical immobilization of enzyme.

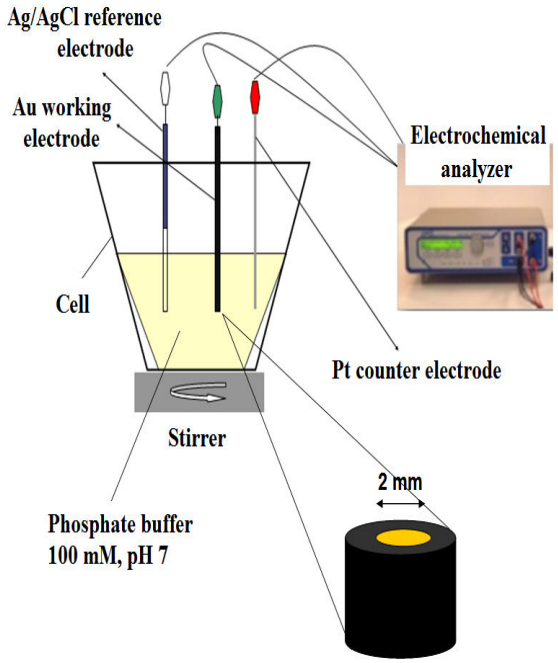

Fig. 1 Experimental setup

TABLE I
ANALYTICAL RESPONSE CHARACTERISTICS OF THE *PGA/PPY/HRP/AU ELECTRODE*

Compound	*Sensitivity (nA/ µM)*	*Linear range (µM)*	*LOD (µM)*	*Response time (s)*	*%RSD*
Phenol	600	16-112	0.087	12	1.8
p-benzoquinone	550	16-160	0.015	6	2.5
p-cresol	400	8-160	0.022	13	3.2
2-chlorophenol	400	4-128	0.114	20	2.7
Catechol	*no response*				

PPy/CNT/HRP/Au working electrode was prepared as follows: nanobiocomposite film was coated onto the surface of the gold working electrode by electrochemical polymerization in three-electrode cell. The polymerization medium contained 5 mL of oxidized carbon CNT solution, 5 mL of 50 mM pH 6.5 citrate buffer including 100 mM pyrrole, 0.6 mg/mL SDS and 0.3 mg/mL HRP. Electropolymerization was conducted at +1V. Enzyme was entrapped into the PPy/CNT film.

III. RESULTS AND DISCUSSIONS

A. Electrochemical Response of the PGA/PPy/HRP/Au Electrode to the Phenolics

Electrochemical batch measurements were carried out in a 100 mM, pH 7 phosphate buffer solution at an applied potential of -50 mV at 600 rpm in three-electrode electrochemical cell. Phenol, catechol, *p*-benzoquinone, *p*-cresol and 2-chlorophenol at the concentration range of 4-200µM were added to the reaction medium, respectively. Typical current-time recording of the biosensor for 2-chlorophenol was shown in Fig. 2. Analytical parameters of the biosensor for the tested phenolic were calculated and presented in Table I.

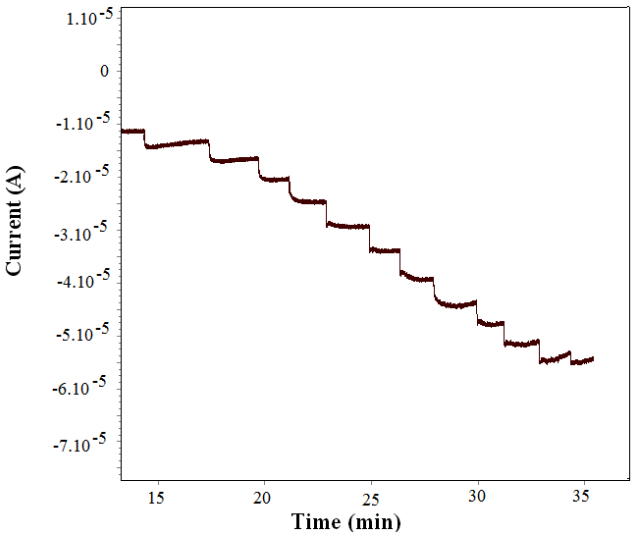

Fig. 2 Biosensor response of PGA/PPy/HRP/Au electrode to 2-chlorophenol additions (4-160µM); applied potential, -50 mV(vs. Ag/AgCl, 3 M NaCl). Reaction medium contained 0.25 mM H_2O_2

The lowest detection limit was found to be 0.015 µM for *p*-benzoquinone and the highest was found to be 0.114 µM for 2-chlorophenol among the tested derivatives. The highest sensitivity was found to be 600 nA/µM for phenol. The sensitivity ranges between 400-600 nA/µM for the phenolics tested. Time to allow the system to come to equilibrium is defined as "response time". The response of the PGA/PPy/HRP/Au working electrode was reached to steady-state current in about 6-20 s for various phenolics. The biosensor response lost only 10% of its initial value at the end of one month. Long storage stability can be attributed to the strong chemical bonding of the enzyme via the Poly(glutaraldehyde) incorporated in the Poly(pyrrole) film.

B. Electrochemical Response of the PPy/CNT/HRP/Au Electrode to the Phenolics

Electrochemical measurements were conducted in the same procedure conducted for the PGA/PPy/HRP/Au electrode. Eighteen phenolic compounds (phenol, catechol, p-benzoquinone, m-cresol, o-cresol, p-cresol, guaiacol, 2,4-dimethylphenol, 2,6-dimethoxyphenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, hydroquinone, 4-acetamidophenol, pyrogallol, 4-methoxyphenol, pyrocatechol, 2-aminophenol) were detected by the biosensor in 100 mM, pH 7 phosphate buffer solution at a working potential of -50 mV (vs. Ag/AgCl). Fig. 3 (not included all the phenolics) illustrates typical amperometric responses for the PPy/CNT/HRP/Au working electrode after the successive addition of phenolic compounds under continuous stirring.

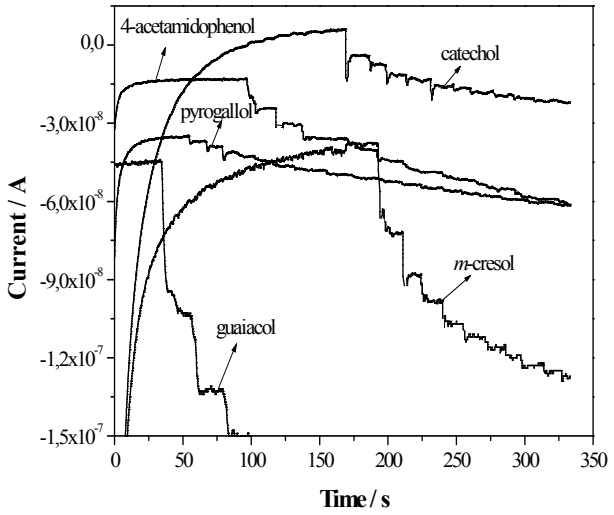

Fig. 3 Current-time recordings of the PPy/CNT/HRP/Au working electrode to increasing 4-acetamidophenol, catechol, pyrogallol, guaiacol and m-cresol concentrations (from the beginning of 1.6µM). Applied potential: -50 mV vs. Ag/AgCl, 3 M NaCl

TABLE II
ANALYTICAL RESPONSE CHARACTERISTICS OF THE PPY/CNT/HRP/AU WORKING ELECTRODE

Compound	*Sensitivity (nA/ µM)*	*Linear range (µM)*	*LOD (µM)*	*Response time (s)*	*%RSD*
Phenol	1	16-144	3.52	2	2.89
p-benzoquinone	3	0.02-0.16	0.027	2	4.43
Hydroquinone	8	16-240	6.42	2	6.5
2,6-dimethoxyphenol	7	1.6-19.2	0.29	2	1.8
2-chlorophenol	8	1.6-8	0.26	2	1.7
3-chlorophenol	6	1.6-12.8	0.2	2	1.1
4-chlorophenol	8	1.6-14.4	0.3	2	1.87
2-aminophenol	40	8-60.8	1.53	2	5.4
4-methoxyphenol	50	1.6-81.6	1.06	2	2.8
Pyrocatechol	8	1.6-446.4	6.27	2	6.7
Guaiacol	9	1.6-9.6	0.3	2	1.92
m-cresol	9	8-20.8	1.5	2	2.84
o-cresol		*No response*			
p-cresol	5	128-832	24	2	2.5
Catechol	2	1.6-8	0.93	2	3.8
4-acetamidophenol	3	1.6-16	1.11	2	2.57
Pyrogallol	1	1.6-22.4	1.24	2	1.2
2,4-dimethylphenol	1	64-240	27.9	2	2.2

Table II summarizes the characteristics of the calibration plots obtained for phenol derivatives. The lowest detection limit was found to be 0.027 µM for *p*-benzoquinone and the highest detection limit was found to be 27.9 µM for 2,4-dimethylphenol among the tested derivatives. The highest sensitivity was obtained from the calibration of 4-methoxyphenol. The sensitivity ranges between 1-50 nA/µM for the phenolics tested. The response of the PPy/CNT/HRP/Au working electrode was rapidly reached to steady-state current in about 2 s for all phenolics tested. This value is obviously shorter than the PGA/PPy/HRP/Au electrode. In the previously reported biosensors, the response time is ranged between 5 and 35 s for various phenolic compounds [9]-[11].

The stability of the PPy/CNT/HRP/Au working electrode was monitored by the measurement of the response for a series of 50 succesive additions of 1.6 µM phenolic to 100 mM, pH 7 phosphate buffer at -50 mV (vs. Ag/AgCl) (Fig.4). Well-defined reduction responses were obtained for all phenolics with relative standard deviations (%RSD) range between 1.1 and 6.7. The biosensor response lost 30% of its initial value at the end of one month. Enzyme was entrapped into the PPy/CNT polymer pores. Long-term stability loose could be attributed to the enzyme leakage from the pores of the polymeric film.

The maximum sensitivity and minimum LOD (minimum detectable concentration) were obtained from the PGA/PPy/HRP/Au electrode for all the phenolics in comparison to the PPy/CNT/HRP/Au working electrode. We reported previously that the use of Poly(glutaraldehyde) in the construction of HRP based electrodes improved the kinetic parameters of the reaction since the enzyme, HRP, was strongly bonded to Poly(glutaraldehyde) with good stability [12]. The incorporation of the active aldehyde groups into the conductive polymeric backbone was successfully achieved to immobilize the enzyme. However, PGA/PPy/HRP/Au electrode responded just five phenolic species while the PPy/CNT/HRP/Au working electrode responded seventeen species.

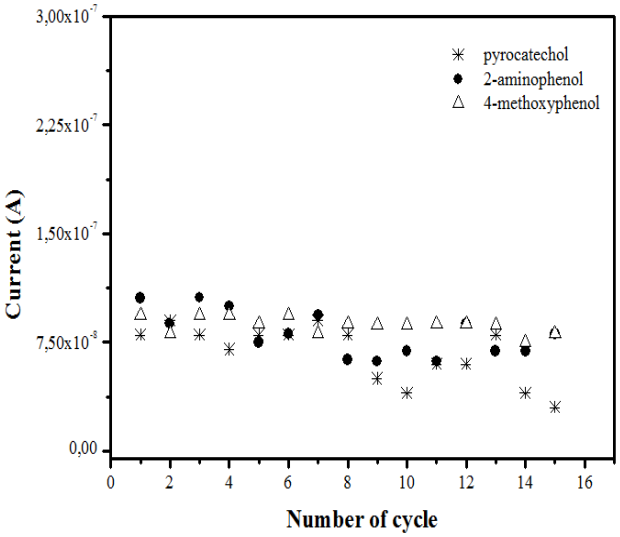

Fig. 4 Operational stability of the PPy/CNT/HRP/Au working electrode obtained from the succesive additions of 1.6 µM phenolic compound. Applied potential: -50 mV vs. Ag/AgCl, 3 M NaCl.

Immobilized enzyme quantity was found to be 3 µg and 6.1 µg for the PGA/PPy/HRP/Au and PPy/CNT/HRP/Au working electrode, respectively according to the procedure which we previously reported [13]. Nanobiocomposite film, involving CNTs, attached higher amount of enzyme than the PGA/PPy/composite film due to CNTs unique structure which supplied larger surface area to immobilize more enzyme. However, results showed that the PGA/PPy/HRP/Au working

electrode was more effective and more selective for phenol detection than the PPy/CNT/HRP/Au working electrode with regard to the measurement analytical parameters.

IV. Conclusion

It was demonstrated that it was possible to modulate the electrical response of HRP-based biosensors by using different modified Poly(pyrrole) based composite film electrodes. Analytical results showed that the higher sensitivities and the lower detection limits were observed for the PGA/PPy/HRP/Au working electrode. Even though the same enzyme was used, each working electrode showed different selectivity to different phenolics depending on electrode configuration. It was clearly understood that not only immobilized enzyme amount but also polymeric film structure affected signals of the prepared biosensors even though the same conductive polmer was used for each electrode.

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