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Preparation of carbon paste electrode containing polyaniline-activated carbon composite for amperometric detection of phenol

H. Arslan[1]*, D. Şenarslan[2], B.S. Çevrimli[3], H. Zengin[4], D. Uzun[1], F. Arslan[1]

[1]*Department of Chemistry, Faculty of Sciences, Gazi University, 06500, Ankara, Turkey*
[2]*Department of Chemistry, Institute of Sciences, Gazi University, Ankara, Turkey*
[3]*Department of Chemical Technology, Ataturk Vocational College, University of Gazi, 06500 Ankara, Turkey*
[4]*Department of Chemistry, Faculty of Arts and Sciences, Gaziantep University, Gaziantep, Turkey*

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In this study, a novel carbon paste electrode was prepared using the salt form of polyaniline (pani)-activated carbon composite sensitive to phenol. Polyphenol oxidase enzyme was immobilized to the modified carbon paste electrode by cross-linking with glutaraldehyde. The amperometric determination is based on the electrochemical reduction of o-quinone generated in the enzymatic reaction of phenol at -0.15 V *vs.* Ag/AgCl. The effects of pH and temperature were investigated and optimum values were found to be 8.0 and 45 °C, respectively. The linear working range of the electrode was 1.0×10^{-6} - 5.0×10^{-5} M, R^2 =0.9819. The storage stability and operation stability of the enzyme electrode were also studied.

Keywords: Phenol, polyphenol oxidase, biosensor, polyaniline (pani), polyaniline activated carbon composite, carbon paste

INTRODUCTION

Carbon paste electrodes are widely used in electroanalysis owing to their low background current, wide potential window, chemical inertness, simple and fast preparation from inexpensive materials. Carbon paste electrodes (CPE) can also be easily modified with electrocatalysts or enzymes by means of simply mixing the modifier into the carbon paste matrix. In addition, the carbon paste electrode offers a renewable electrode surface [1].

A large variety of phenolic compounds exists. Some of them may have harmful effects for the health [2]. Their accurate determination is of great importance due to their toxicity and persistency in the environment, and the detrimental effect of phenols on human health requires a strict directive for the identification and quantification of such compounds [3-5].

For phenolic compounds determinations, polyphenol oxidase (also known as tyrosinase, EC 1.14.18.1), which is a copper containing enzyme, is used [6]. This enzyme catalyses phenol oxidation and *o*-quinone is the product of the enzymatic reaction. This is accomplished in two reaction steps. In the first step, tyrosinase oxidizes phenol into the corresponding catechol. In the second step, the catechol is oxidized into *o*-quinone. Amperometric reduction of the generated o-quinone is then used as the quantification method [7, 8].

A variety of methods for the immobilization of tyrosinase with an electrochemical transducer have been reported such as cross-linking on the surface of electrodes [9-11], incorporation within a carbon paste matrix [12, 13], entrapment in polymer films [6, 14, 15].

In this study, a novel carbon paste electrode using the salt form of polyaniline (pani)- activated carbon composite sensitive to phenol, was prepared. Polyphenol oxidase enzyme was immobilized on the carbon paste electrode containing polyaniline-activated carbon by cross-linking with glutaraldehyde. The optimum working conditions of the modified carbon paste (MCPE) with respect to the substrate concentration, the pH and temperature were investigated. The storage stability and operation stability of the biosensor were investigated.

Materials and methods

Apparatus: The electrochemical studies were carried out using a CHI 660B electrochemical workstation with a three-electrode cell. The working electrode was a carbon paste (diameter of 1.0 cm, length of 5 mm) Teflon electrode. The auxiliary and reference electrodes were Pt wire and Ag/AgCl electrode (3 M KCl), respectively. The pH values of the buffer solutions were measured with an Orion Model 5 Star pH/ion meter. Temperature control was achieved with a Grant W14 thermostat.

Chemicals: Polyphenol oxidase (EC 1.14.18.1, with an activity of 10 unit/mL) and phenol were purchased from Sigma. Graphite powder and nujol were supplied by Merck and Sigma, respectively. All other chemicals were obtained from Sigma. All

* To whom all correspondence should be sent.
E-mail: halit@gazi.edu.tr; dr.halitarslan@gmail.com

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solutions were prepared using double distilled water.

Preparation of modified carbon paste electrode (MCPE)

The carbon paste was prepared with 2 mg polyaniline-activated carbon composite by thoroughly mixing 100 µL of nujol with 0.15 g of graphite powder in a mortar [16]. Polyaniline-activated carbon composite was synthesized according to Zengin and Kalaycı [17]. For the preparation of the carbon paste electrode a glass tube (diameter of 1.0 cm, length of 0.5 cm) was filled with the paste. Height of the paste in the tube was 0.5 cm. The electrode surface was smoothed on a paper to produce a reproducible working surface. Electric contacts were made by platinum wire. 75 µL of polyphenol oxidase enzyme (10 unit/mL), 1 mg of bovine serum albumin, 50 µL of 0.1M phosphate buffer of pH 8.0 and 30 µL of 2.5% glutaraldehyde were dropped upon the carbon paste electrode containing polyaniline - activated carbon composite. The electrode was dried at room temperature and washed with buffer solution (0.1 M phosphate buffer, pH 8.0,) several times in order to remove the non-immobilized excess enzyme and glutaraldehyde. The electrode was kept in a refrigerator at 4° C in phosphate buffer when it was not in use.

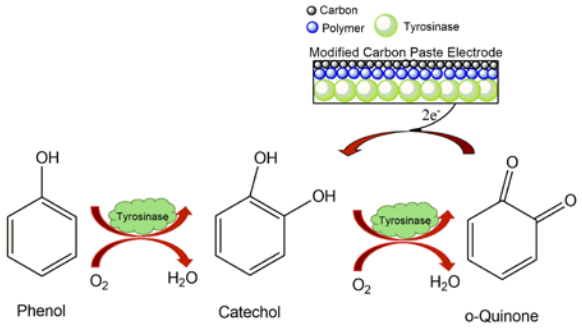
Electrochemical measurements

The quantification of phenol was achieved *via* electrochemical detection of the enzymatically released *o*-quinone. The modified carbon paste electrode (MCPE) was immersed into the phosphate buffer (0.1 M) of pH 8.0. The solution contained 0.1 M sodium perchlorate as supporting electrolyte. The electrode was brought to equilibrium by keeping at -0.15 V (*vs*. Ag/AgCl electrode (3 M KCl)). Steady current (i_a) was recorded. Phenol solution was added to the cell and the system was stirred. The currents (i_b) obtained at -0.15 V were recorded. The current values ($\Delta i = i_b - i_a$) were plotted against the phenol concentrations.

RESULTS AND DISCUSSION

In this study, we reported a new amperometric biosensor for the determination of phenol. Polyphenol oxidase (tyrosinase) enzyme was immobilized onto a carbon paste electrode containing polyaniline-activated carbon by cross-linking with glutaraldehyde. The amperometric determination is based on the electrochemical reduction of o-quinone generated in the enzymatic reaction of phenol at -0.15 V *vs*. Ag/AgCl. Reaction scheme 1 shows the phenol determination. According to this scheme, a biochemical reaction occurs between phenol in solution and tyrosinase enzyme which are immobilized onto the carbon paste electrode containing polyaniline-activated carbon. Firstly, phenol is oxidized to catechol. Then, the catechol is oxidized into *o*-quinone. By taking the electron, oxygen is reduced to H_2O. Phenol determination was made by measuring the reduction current to *o*-quinone on the electrode surface.

The parameters affecting the performance of the biosensor and the optimum working conditions were investigated.



Scheme 1. Reaction scheme of phenol determination

Working potential

After preparing the modified carbon paste electrode (MCPE), the electrochemical reduction of o-quinone generated in the enzymatic reaction of phenol was carried out at different potentials (-0.07, -0.11, -0.15, -0.19 V) (Fig. 1). In all cases, as shown in Figure 1, the highest current differences and correlation coefficient were obtained at -0.15 V. Therefore, -0.15 V was used as working potential in the following studies.

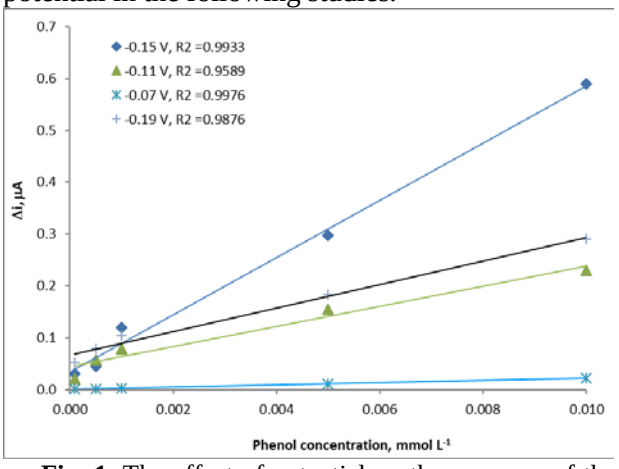


Fig. 1. The effect of potential on the response of the modified carbon paste electrode to *o* quinone (at 25 °C, 0.1 M pH =8.0 phosphate buffer, -0.15 V operating potential).

Determination of optimum pH

Since enzyme activity is dependent on the ionization state of the amino acids in the active site,

pH plays an important role in maintaining the proper conformation of an enzyme. The effect of pH on the response to phenol of MCPE was determined in 0.1 M phosphate buffer, in the pH range 6.0-10.0. The measurements were performed at a constant phenol concentration of 1.0×10^{-5} M. Figure 2 shows that the maximum response was obtained at pH 8.0. For MCPE, pH values different from 8.0 were employed in the literature (pH 7.5; 6.5) [14, 18]. In another study by Arslan *et al*. the optimum pH was found to be 8.0 [15].

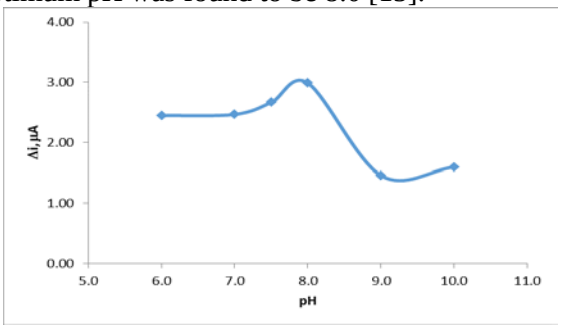


Fig. 2. Effect of pH on the response of MCPE (at 25 °C, 1.0×10^{-5} M phenol, -0.15 V operating potential).

Determination of optimum temperature

Enzymes are known to be sensitive to changes in temperature. The relationship between reaction rate of an enzyme and temperature is exponential. The temperature influence on the response of phenol MCPE was tested between 20ºC and 60ºC at pH 8.0 using constant phenol concentration of 1.0×10^{-5} M. As seen from the Figure 3, the current difference increases with temperature up to 45ºC and decreases afterwards. The highest electrode response was obtained at 45ºC. For MCPE, temperature values different from 45ºC were employed in literature (30, 40, 21 ºC) [9, 15, 18]. The study was carried out at 25ºC due to the difficulties involved in working at 45 °C.

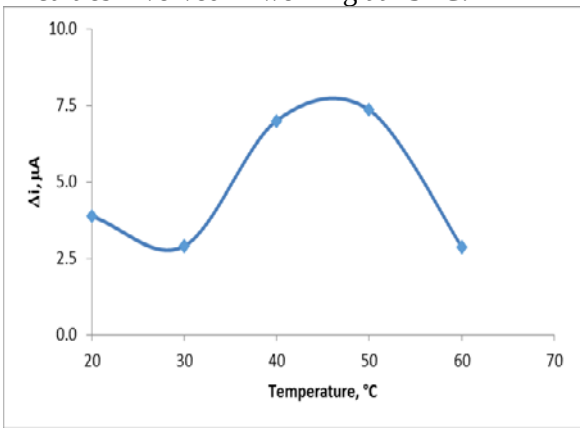


Fig. 3. The effect of temperature on the response of MCPE (at pH 8.0, 1.0×10^{-5} M phenol at -0.15 V operating potential).

Effect of substrate concentration on response of MCPE and calibration curve

The effect of substrate concentration on the reaction rate, catalyzed by immobilized PPO, was studied using varying concentrations (1.0×10^{-6} – 1.0×10^{-3} M) of phenol (Figure 4). The linear working range of the electrode was 1.0×10^{-6} - 5.0×10^{-5} M, R^2 =0.9819 (Figure 5).

It is seen that the linearity of graphs is highly satisfactory and they could be used for the quantitative determination of phenol. The detection limit of the biosensor was 5.0×10^{-7} M and the response time of the biosensor was 200 s.

Kinetic parameters $I_{max(app)}$ and $K_{m(app)}$ for the enzyme biosensor were calculated as 3.47 μA, 0.69 mM respectively. Km values for immobilized polyphenol oxidase presented in the literature are 100, 0.67 mM [14, 19]. This was attributed to the fact that the polymer used and the type of immobilization were different.

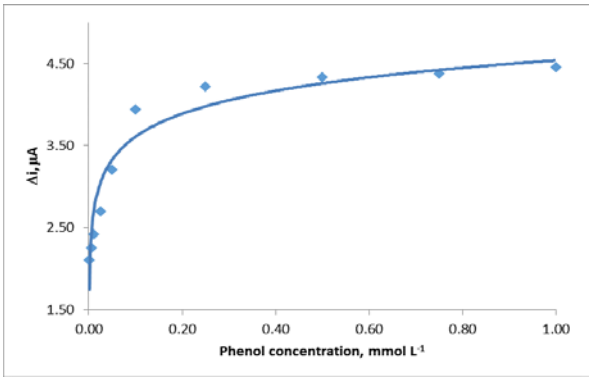


Fig. 4. The effect of phenol concentration upon the amperometric response of MCPE (in pH 8.0 phosphate buffer and at a -0.15 V operating potential, 25 °C).

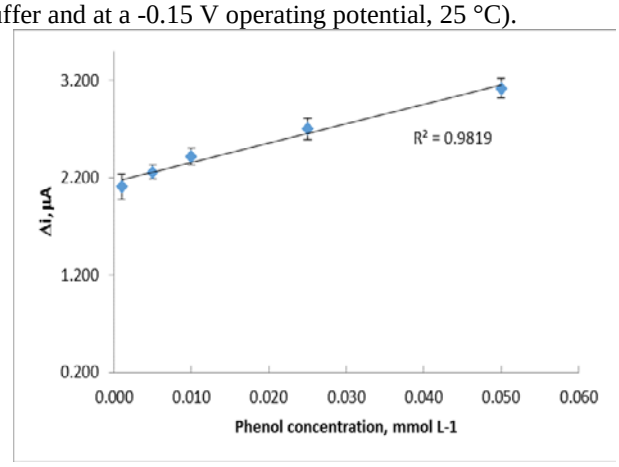


Fig. 5. The calibration curve of the MCPE (in pH 8.0 phosphate buffer and at a -0.15 V operating potential, 25 °C)

The operational stability of the MCPE

The operational stability of MCPE was studied by performing the activity assay (under optimum conditions) 15 times in the same day (Figure 6).

The relative standard deviation obtained after 15 measurements at a constant phenol concentration of 1.0×10^{-5} M was found to be 2.75%.

Storage stability of MCPE

The activity assay was applied within 35 days to determine the storage stability of the immobilized enzyme. As shown in Figure 7, during the 35 days, the response of MCPE decreased. An activity loss of 53 % was observed on the 35th day.

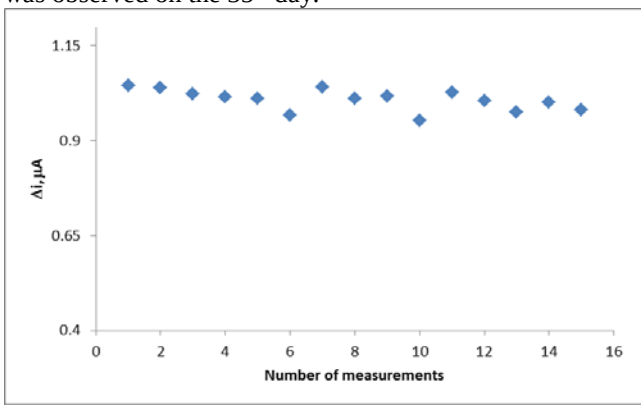


Fig. 6. Operational stability of MCPE in pH 8.0 phosphate buffer, at a -0.15 V operating potential, 25 °C.

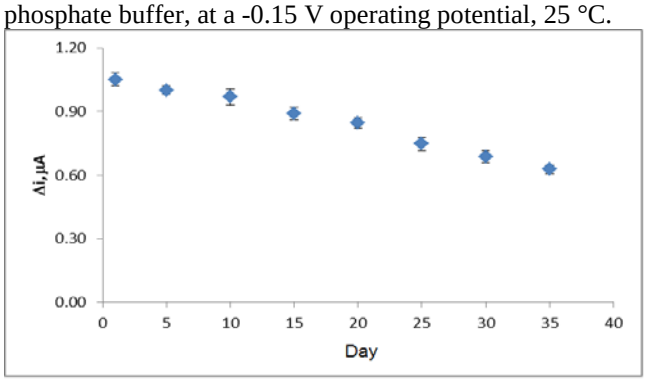


Fig. 7. Storage stability of MCPE (in pH 8.0 phosphate buffer, at -0.15 V operating potential, 25 °C and 1.0×10^{-5} M phenol concentration).

Interference effects

Several cations found in wastewater, such as Cd^{2+}, Pb^{2+}, Sn^{2+}, As^{3+}, As^{5+}, Cr^{3+}, Cr^{6+}, Sb^{3+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, were studied for any interfering effect on the analysis of phenol. 1.0×10^{-2} M - 1.0×10^{-5} M concentrations of cations were added. It was observed that Cd^{2+}, Pb^{2+}, Sn^{2+}, As^{3+}, As^{5+}, Cr^{3+}, Cr^{5+}, Sb^{3+}, Mn^{2+}, Co^{2+}, and Ni^{2+} had no interfering effects on the analysis of phenol. However, interfering effect of copper (1.0×10^{-2} - 1.0×10^{-5} M) on the analysis of phenol was observed.

CONCLUSION

In this study, polyphenol oxidase was successfully immobilized on a polyaniline (pani)-activated carbon composite. The experimental results showed clearly that the biosensor exhibited good performance in the determination of phenol. It was found that operational stability and long-term storage stability of the phenol biosensor were good.

Phenol biosensor prepared in this study is useable in a wide concentration range 1.0×10^{-6} - 5.0×10^{-5} M (R^2 =0.9819). It has a very low detection limit (5.0×10^{-7} M) and an acceptable response time for a biosensor (200 s). It gives perfect reproducible results (the relative standard deviation is 2.75 % after 15 measurements). Also it has good storage stability (gives 47 % of the initial amperometric response at the end of the 35th day). The $K_{m(app)}$ and $I_{max(app)}$ values of polyphenol oxidase enzyme immobilized in polyaniline (pani)- activated carbon composite are 0.69 mM and 3.47 μA, respectively. MCPE proposed in this study is easy to prepare and highly cost-effective.

***Declaration of interest**: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.*

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ПРИГОТВЯНЕ НА ВЪГЛЕРОДЕН ПАСТООБРАЗЕН ЕЛЕКТРОД, СЪДЪРЖАЩ ПОЛИАНИЛИН-АКТИВИРАН ВЪГЛЕРОДЕН КОМПОЗИТ ЗА АМПЕРОМЕТРИЧНО ОПРЕДЕЛЯНЕ НА ФЕНОЛ

Х. Арслан[1]*, Д. Шенарслан[1], Б.С. Чеврimли[2], Х. Зенгин[3], Д. Узун[1], Ф. Арслан[1]

[1] Департамент по химия, Научен факултет, Гази университет, 06500, Анкара, Турция
[2] Департамент по химична технология, Професионален колеж Ататюрк, Гази университет, 06500, Анкара, Турция
[4] Департамент по химия, Факултет по наука и изкуства, Газиантеп университет, Газиантеп, Турция

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(Резюме)

Приготвен е нов въглероден пастообразен електрод с използване на полианилинов активиран въглероден композит, чувствителен към фенол. Полифенол оксидазен ензим е имобилизиран към модифициран въглероден пастообразен електрод чрез омрежване с глутаралдехид. Амперометричното определяне се основава на електрохимичната редукция на *o*-хинон, генериран от ензимната реакция на фенол при -0.15 V спрямо Ag/AgCl. Изследвано е влиянието на pH и температурата, като оптималните стойности са съответно 8.0 и 45 °C. Линейният работен интервал на електрода е 1.0×10^{-6} - 5.0×10^{-5} M, $R^2 = 0.9819$. Изследвани са стабилността при съхранение и работната стабилност.