Different Part of Mushroom Tissue Used as Modifier in Carbon Paste Sensor

Karbon Pasta Biyosensöründe Dönüştürücü Olarak Kullanılan Mantar Dokusunun Farklı Bir Yönü

Research Article

Nevila Broli* and Majlinda Vasjari
Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania

ABSTRACT

An enzymatic carbon paste electrode for determination of phenolic compounds is developed. The electrode material was prepared by mixing the monodisperse graphite powder with paraffin and with crude mushroom tissue which contains polyphenol oxidase (PPO) in its living environment. The ratio between components (carbon - tissue - paraffin), was investigated using SCV (Stare Case Voltammetry) techniques in 0.1 M sodium phosphate buffer, pH 7, scan rate 50 mV/s. The derivative voltammogrammes are used to evaluate the biosensor response. The analytical performance was closely related with the carbon powder granulometry. The optimal results regarding the background current and sensitivity (1.57 mA/ppm) were obtained when carbon powder granulometry was 0.09-0.071 mm. It was also studied the activity of the PPO taken from four separated sections of mushroom body. Kinetic of enzymatic reaction resulted according to the Michaels–Menten mechanism. The biosensor response was tested toward different phenolic compounds. The best signal regarding the sensitivity of biosensor (S=2.04 mA/ppm), correlation coefficient (R^2= 0.9997) and detection limit (0.7 ppm) was obtained for hydroquinone and the other studied compounds are listed in these order: hydroquinone > catechol > phenol > m-cresol > 4-chlorophenol > p-cresol > 4-nitrophenol > 3-nitrophenol.

Key Words
Phenolic compound, carbon paste biosensor, PPO, crude tissue, Voltammetry

ÖZET

Fenolik bileşiklerin belirlenmesi için enzimatik bir karbon elektrot macunu geliştirilmiştir. Elektrot malzemesi parafin ve canlı ortamda polifenol oksidaz (PPO) içeren ham mantar doku ile birlikte tek dağılımlı (monodisperse) grafit tozunun karıştırılması ile hazırlanmıştır. Bileşenleri arasındaki oran (karbon - doku - parafin), 0.1 M sodyum fosfat tamponu, pH 7, ve 50 mV/s tarama hızında SCV (Stare Case Voltammetry) teknikleri kullanılarak araştırılmıştır. Türev voltamogramlar biyosensör yanıtını değerlendirmek için kullanılır. Analitik performans karbon tozu granülometrisi ile yakından ilgilidir. Karbon tozu granülometrisi 0,09-0,071 mm iken, arka akım ve hassasiyeti (1.57 mA / ppm) ile ilgili olarak en iyi sonuçlar elde edildi. Ayrıca mantar gövdesinin ayrılmış dört bölümünden alınan PPO aktivitesi incelenmiştir. Enzimatik reaksiyonun kinetiği Michaels-Menten mekanizmasına göre sonuçlanmıştır. Biyosensör yanıtı, farklı fenolik bileşiklere karşı test edilmiştir. Hidrokinon için biyosensör duyarlılığı ile ilgili en iyi sinyal (S = 2.04 mA / ppm), korelasyon katsayısı (R^2 = 0,9997) ve algılama sınırı (0.7 ppm) elde edilmiştir ve diğer çalışılan bileşikler şu sırada verilmektedir: hidrokinon> katekol > fenol> m-kresol> 4-klorofenol> p-kresol> 4-nitrofenol> 3-nitrofenol.

Anahtar Kelimeler
Fenolik bileşik, karbon pasta biyosensör, PPO, ham doku, voltametri

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Correspondence to: Nevila Broli, Department of Chemistry, Faculty of Natural Sciences, Bul. "Zogu I", Tirana, Albania
Tel: +355682092552 Fax: +90 312 299 21 63 E-Mail: nevila.parroj@fshn.edu.al; nevilabroli@yahoo.com

INTRODUCTION

Determination of phenolic compounds is essential because of their widespread use and potential toxicity to organisms. Phenol is reported to be carcinogenic and exposure to phenol results in several symptoms such as convulsions, dizziness and irregular respiration [6]. In the food industry, phenols are of interest because they are essential compounds of fruit juices, beer, and wines [2]. Prolonged oral or subcutaneous exposure causes damage to the lungs, liver, kidney and genitourinary tract [12]. Since many phenolic compounds can cause bad taste and undesirable odor and are highly toxic and hazardous to human health, their analysis at low concentrations is very important [6, 2, 12]. As the manufacture and use of phenols requires qualitative and quantitative control, a wide variety of methods have been developed to determine phenolic compounds. For phenol determination various spectrometric and chromatographic methods are in common use Instead of those conventional methods, electrochemical methods based on enzymatic biosensors have been developed [7]. Classical methods for immobilizing enzymes are physical adsorption, entrapment and covalent cross-linking. Although entrapment of purified enzymes in matrices has been performed, enzymes often fail to retain their native stability and activity upon immobilization; as a result, most of these immobilizing methods were neither simple nor stable [15]. Recently the preference for using vegetable or plant tissue instead of purified enzymes, directly without any pretreatment is reported. This group of biocatalyst materials maintains the enzyme of interest in its natural environment [6, 2, 3]. The cell represents the most suitable milieu for enzymes, giving them a high stability and high activity due to the presence of coenzymes and activators which are often required. In comparison to the isolated enzyme, which can lose its activity during isolation or immobilization plant tissues provide a novel and cost effective approach to the construction of biosensors [3, 10, 14].

Carbon paste electrodes (CPEs) are widely applicable in electrochemical studies due to their low background current (compared to solid graphite or noble metal electrodes), low cost, feasibility to incorporate different substances during the paste preparation (in the case of modified carbon paste electrodes), easy preparation and simple renewal of their surface and possibilities of miniaturization. The influence of carbon powder granulometry on the biosensors response is studied here. Carbon paste electrodes modified with different plants tissue as source of PPO has been reported [12, 1, 13]. PPO are widely distributed in most known fruits and vegetables. The concentration of this enzyme in plants depends on the species, cultivation, age and maturity [8]. In this study we report the changes of the carbon paste electrode analytical performance depending on the crude tissue taken from different morphological part of the same mushroom plant.

EXPERIMENTAL

Electrochemical experiments were carried out in a stirred electrochemical cell containing 15 mL of phosphate buffer solution 0.1 M. All chemicals used were of analytical grade (Merck) and used without any further purification. Phosphate buffer solution was prepared by mixing suitable amounts of 0.1 M $K_2HPO_4.3H_2O$ and KH_2PO_4, K_2HPO_4, KCl. Stock solutions of phenol, catechol , hydroquinone, p-cresol, m- cresol, 4-chlorophenol, 3-nitrophenol, 4-nitro phenol, 3-aminophenol (www.schuchardt.de) were prepared by dissolving the right amount of pure substance in distilled water. Electrochemical analyzer (MEC-12B) using a three electrode system was used to record all SC voltamogramms. Working electrode was a homemade carbon paste biosensor modified with mushroom tissue. Different granulometry of carbon powder obtained through mechanically grinding the pencil leads (www.Sanfordcorp.com) and using a series of sieves was studied. The crude tissue taken from different morphological part of the mushroom plant was used as source of PPO purchased from a local market as fresh culture vegetable was investigated. The reference and auxiliary electrode were Ag/AgCl and a platinum wire respectively. The voltammograms were recorded from E_i = -0.4 V to E_f = 1.0 V, in 0.1 M buffer solution (pH= 7), scan rate 50 mV/s. In each case the background voltammogram was firstly recorded and then the addition of phenolic standard solution was introduced into

the cell. All experiments were conducted at room temperature.

Biosensors Construction

The electrode material is prepared using the crude tissue of mushroom taken exactly from one morphological part of the plant, (Figure 1 a/b/c/d), the graphite powder with the same granulometry, and paraffin mixed together and homogenized. Firstly the graphite powder and paraffin were mixed and then the mushroom

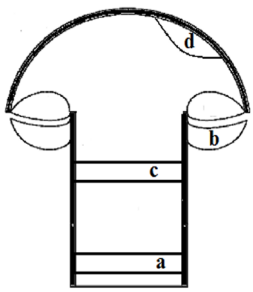

Figure 1. Different part of mushroom used as sensor modifier: a) bottom of stem, b) gill, c) upper of stem, d) cap.

tissue was added and mix for at least 20 min to obtain a homogenous paste. The paste was stored in refrigerator at 4°C for 24 hour. A portion of each mixture (about 1.1 g) was packed into the tip of a 1 mL plastic syringe which contains a copper wire for the external electric contact. The surface of the working electrode was smoothed using a clean glass surface before the measurements. Unmodified carbon paste electrode was prepared in the similar way without using biological modifier.

RESULTS AND DISCUSSION

The effect of incorporated PPO to sensor response was tested by comparing the voltammograms obtained in the same conditions by both electrodes unmodified carbon paste (CPE) and modified carbon paste electrodes (MCPE). In this experiment the crude tissue was taken from d section of the mushroom plant. The immobilized PPO is a copper-containing enzyme capable to catalyze two different types of reactions in the presence of molecular oxygen: the hydroxylation of mono-phenols to o-diphenols and the oxidation of o-diphenols to o-quinones [14]. Another advantage of this system is that the o-diphenols electrochemically produced are active as substrate for the enzyme thus an amplification of the analytical signal was expected [4].

Cyclic voltammograms recorded using unmodified carbon paste electrode (CPE) and modified carbon paste electrode with crude tissue (MCPE) in different phenol concentrations, in phosphate buffer 0.1 M, pH 7 and scan rate 50 mV/s. are shown in Figure 2. In unmodified electrode the analytical signal of phenol oxidation occurs at 0.77 V versus Ag/AgCl. In the case of modified electrode the potential of oxidation was shifted to less positive values 0.75 V. It was expected to obtain the higher oxidation current when the cell concentration of phenol was increased. While this was obtained using both kinds of sensors the difference in sensitivity was noticed. The calibration graphs up to 10 ppm phenol concentration are obtained using modified

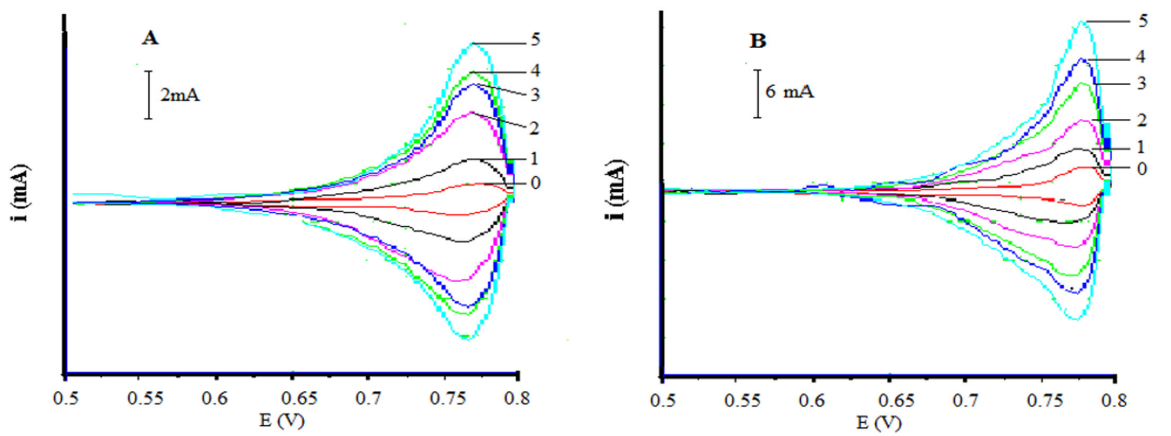

Figure 2. Cyclic voltammograms in 0.1 M phosphate buffer pH 7, scan rate = 50 mV/s. using CPE (A) and MCPE (B), in different phenol concentrations (0) buffer, (1) 1.4 ppm, (2) 3.3 ppm ; (3) 6.6 ppm ; (4) 9.4 ppm; (5) 9.4 ppm; using CPEM in the same phenol concentrations.

Table 1. Optimization of composite biosensor parameters.

Parameters	Range studied	Optimal value
Paraffin (μL)	270-320	280
Mushroom tissue (g)	0.05-0.2	0.1
pH	4-9	7
Scan rate (mV/s)	50-200	50
Potential range/V(CV)	-0.4 to 1.0	0.7

and unmodified sensor. Sensitivities were 0.98 mA/ppm and 0.33 mA/ppm respectively. In the higher concentration the behavior of both sensors does not remain linear.

The components ratio in the performance of the working electrode material was studied. Using a fixed amount of graphite powder 1.000 g the amount of paraffin and mushroom tissue were varied. The amount of paraffin was varied from 300, 280, 250, 230 μL and amount of crude tissue varied as 0.05, 0.1, 0.15, 0.2 g. In each case the response of the biosensor was recorded in 3.3 ppm phenol in different pH and scan rate as are shown in Table 1. Based on the performance of the biosensor related to the shape of the voltammograme, background current, correlation coefficient of the calibration graphs and sensitivity the optimal ratio between components of the electrode material were found. The modified carbon paste prepared by homogenization of 280 μL paraffin, 0.1 g mushroom tissue and 1.0 g graphite powder resulted with the best analytical performance (sensitivity 0.99 mA/ppm, R^2 = 0.9902). The highest signal was found at pH 7 using scan rate 50 mV/s. It was expected because it corresponds to the optimal pH of enzyme activity. The optimal modification of the carbon paste and the optimal experimental condition are used in all the following experiments.

As it is reported in other studies and is conformed in this study the performance of carbon paste electrode is affected by the components of the electrodic material and the ratio between them [1]. In the case of carbon paste electrode the particles size of graphite powder should be taken in consideration because the conductivity of graphite is conditioned by its structure. So while the larger particles would provide better conductivity and non-homogenous carbon paste composition, with finer particles the homogeneity would be improved and their conductivity would be decreased. For this reason, this paper is studying the influence of particle size of graphite powder on modified carbon paste electrode.

Using a fixed ratio between components in each carbon paste (1.000 g graphite powder /300 μl paraffin/0.100 g mushroom tissue), different

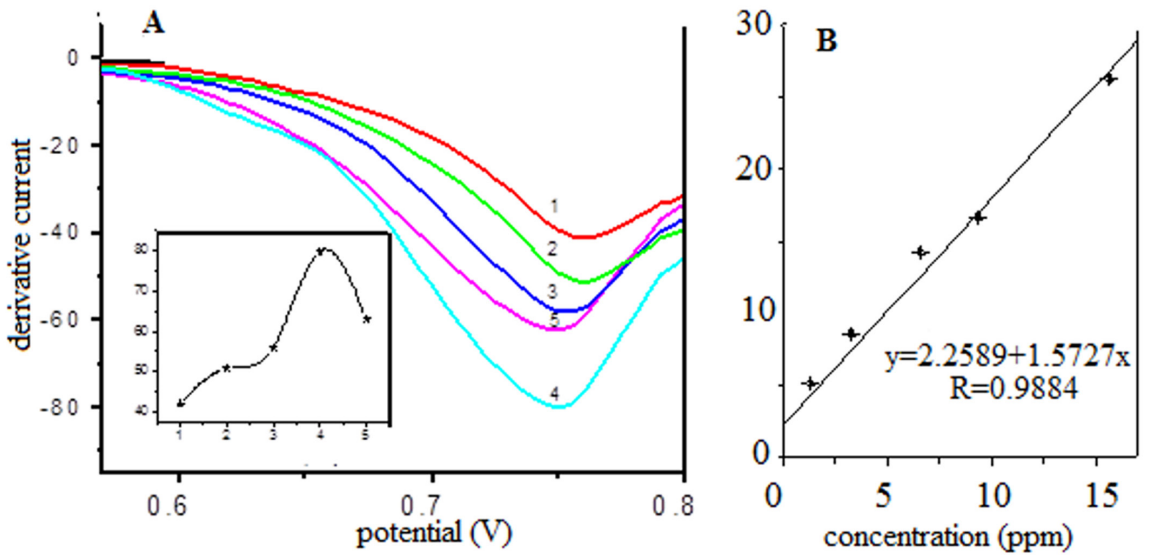

Figure 3. A) Derived voltammograms of MCPE electrode prepared with different particles size of graphite powder (1) 0.5-0.2 mm; (2) 0.2-0.16 mm; (3) 0.16-0.09 mm; (4) 0.09-0.071 mm; (5) 0.071-0.056 mm, with phenol concentration 3.3 ppm, in phosphate buffer 0.1 M (pH 7), scan rate 50 mV/s, B) Calibration curve obtained at potential 0.78 V for 0.09-0.071 mm granulometry.

particles size of graphite powder (0.5-0.2, 0.2-0.16, 0.16-0.09, 0.09-0.071, 0.071-0.056 mm) is used in each case. Figure 3A shows the voltammograms recorded for MCPE electrode prepared with different particles size of graphite powder in phenol concentration 3.3 ppm, pH = 7 buffer solution.

By comparing the analytical signal obtained using different granulometry of carbon powder it was noticed that the peak high pass through a maximum (Figure 3A). This results suggested that the current may be partly controlled by the conductivity and partly by the homogeneity of the carbon paste. Decreasing the granulometry of the carbon powder from 0.5 mm to 0.09 mm the current was increased because of improvement of homogeneity of the paste. Highest signal was obtained using 0.09-0.071 mm particle size of carbon powder. Fine granulometry (0.071-0.056 mm) was affected by the poor conductivity and the current was decreased. Also the same was observed in sensitivity of the carbon paste electrode: larger fraction particles size of graphite powder the sensitivity of MCPE is lower and with decreasing of particles size the sensitivity was increased. The best response regarding background current and sensitivity (S= 1.57 mA/ppm) was obtained using the particles size 0.09-0.071 mm of graphite powder (Figure 3B).

It is well known and in several papers is reported [8], the distribution of PPO enzymes activity is not uniform in each morphological part of mushroom. Here there are experimented modified carbon paste electrodes using crude tissue taken from different sections in accordance to the mushroom schematic shown in Figure 1.

The electrochemical behavior of experimented biosensors was studied based on the

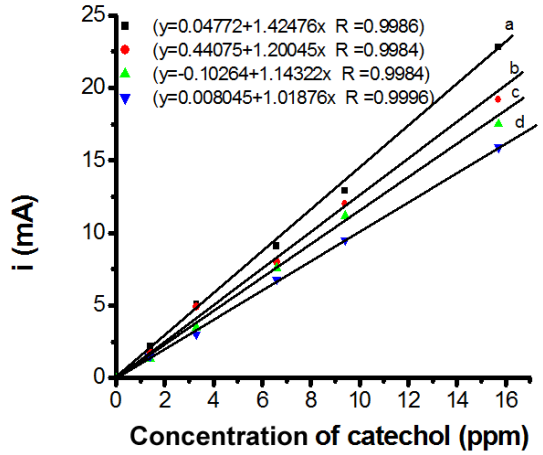

Figure 4. Calibration curves of CPEM using a.b.c.d section as modifier, E = 0.4 V, scan rate 50 mV/s.

voltammogrames and corresponded calibration graphs obtained in each case. The measurement was performed using catechol as a substrate in phosphate buffer 0.1 M, pH 7. In Figure 4, it is obviously shown that the source of modifier resulted in changes in the calibration curves sensitivity. The stalk tissue (section a) appears to contain more active enzyme than other studied morphological parts.

The sensitivity calculated in the linear range decreased in this order: section (a) > section (b) > section (c) > section (d). The results obtained here showed that the enzyme activity is determined by its living environment and is very important the incorporation step into the carbon paste do not affect in its activity.

Volammograms obtained by MCPE under the optimized experimental conditions using catechol as substrate shown that with increasing catechol concentration the response of the MCPE increases (Figure 5A).

Table 2. Analytical parameters of biosensor in various phenolic compounds.

Analyte	Sensitivity (mA/ppm)	R^2	Detection limit (ppm)	Standard deviation	Relative response (%)
Hydroquinone	2.04	0.9997	0.75	0.31	100
Catechol	1.27	0.9986	1.77	0.44	62
Phenol	1.12	0.9541	9.56	2.29	55
m-Cresol	0.98	0.9659	4.52	1.16	48
4-Chlorophenol	0.95	0.9979	1.37	0. 4	47
p-Cresol	0.79	0.9650	5.58	1.39	39
4-Nitrophenol	0.77	0.9921	2.63	0.64	37
3-Nitrophenol	0.70	0.9405	6.53	1.64	34
4-Aminophenol	no response	-	-	-	-

When the concentration of catechol is higher than 25.5 ppm a platform is observed, showing a characteristic of the Michaels-Menten kinetic mechanism (Fig 5B). Supposing that oxidation of catechol could be happening according to Michaels-Menten mechanism Km constant is calculated. Using the Lineweaver–Burk equation Km value is Km=8.45 ppm (Figure 5C). The linear response is from 3.3 to 25.5 ppm with a correlation coefficient of 0.9956 (n = 7) and a detection limit of 1.8 ppm. The response of the MCPE to phenol, 4- clorophenol, hydroquinone, catechol, p-cresol, m-cresol, 4-aminophenol, 3-nitrophenol, 4- nitrophenol was investigated. The SCV technique in phosphate buffer 0.1 M, pH 7, and E= (-4.0 – 1.0 V) was used. No response was obtained towards 4-aminophenol. Table 2 summarizes the characteristics of the calibration plots for the studied phenol derivatives, as well as the sensitivity, detection limit and standard deviation.

The detection limit ranged between 0.75 and 9.56 ppm for the tested phenol derivatives. The different sensitivities varied between 0.70 – 2.04 mA/ppm for the tested phenolic can be related to the formation of o-quinones during the enzymatic reaction [11]. The maximum sensitivity was found to be 2.04 mA/ppm for hydroquinone. The difference in sensitivity between each mono-phenolic compound might be depended on the hydrophobic characteristics of the immobilization matrix [9]. The MCPE biosensor showed relatively high sensitivity for catechol (S= 1.27 mA/ppm). This can be related with the presence of –OH group of catechol in orto position which enhances oxidation of the o-diphenol to quinone by PPO enzyme. Table 2 shows the relative response relationship of the MCPE biosensor in the same experimental conditions in the solutions of catechol, hydroquinone, 4-chlorophenol, phenol, p-Cresol, m-cresol, 4-nitrophenol, 3-nitrophenol, and 4-aminophenol. The values are calculated considering the response of MCPE in hydroquinone 100%. The different responses between those

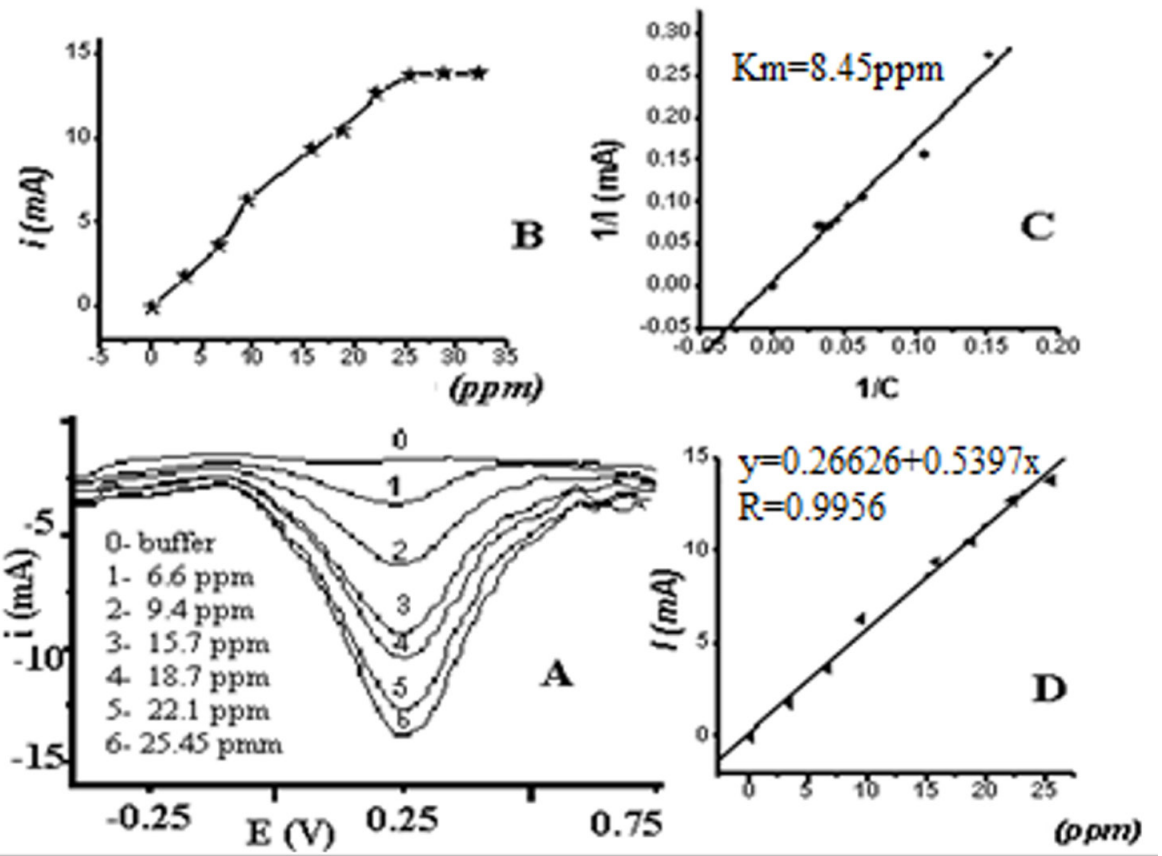

Figure 5. A) Voltammograms of MCPE obtained in different catechol concentration (pH 7.0). B) Current obtained vs. catechol concentration. C) The regression of the sensing experimental data by Michaelis-Menten model. D) Sensor calibration in linear zone.

phenolic compounds are due to the different substituent groups and their position. Therefore, good signals were obtained to –OH groups in para and –ortho position that confer a higher reactivity characteristic to the compounds.

CONCLUSIONS

In this study it is shown that the analytical performance of carbon paste phenolic biosensors modified with crude tissue of mushroom plant is closely related with the granulometry of the carbon powder and the ratio between paste components. Morphological part of the mushroom as source of PPO should be carefully chosen in order to improve analytical performance of the phenolic biosensor. From this study granulometry of 0.09-0.071 mm would be suggested to prepare carbon paste electrode for determination of phenolic compounds. Kinetics of oxidation enzymatic reaction resulted according to the Michaels–Menten model with characteristic constant Km=8.45 ppm.

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