PHENOLIC BIOSENSOR BASED ON CARBON PASTE ELECTRODE MODIFIED WITH CRUDE TISSUE

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Abstract

The biosensors based on carbon paste electrode modified with the crude tissues of banana, apple, mushroom, potato, pear plant as polyphenol oxidase source for quantification of phenolic compounds were studied. The ratio between components (carbon - tissue - paraffin), was investigated to optimize the electrode material with better electro analytical performance using SCV (Stare Case Voltametry) techniques in 0.1 M sodium phosphate buffer, pH 7, scan rate 50 mV/s. The best results of CPEB sensor regarding the background current, sensitivity and correlation coefficients were obtained when the mixture of 1g graphite powder/ 300 εl paraffin and 0.1 gr tissue was used. The electrochemical behavior of carbon paste electrode modified with different kind of crude tissues is compared with electrochemical behavior of unmodified electrode. Relative response of biosensor modified with crude tissue of banana (CPEB) and apple (CPEA) to different phenolic compounds is studied. The CPEB biosensor shows high sensitivity to hydroquinone (S=3.25mA/ppm) and lowest detection limit (1.02 ppm) to phenol. CPEA biosensor shows better sensitivity for 4-clorphenol (S=1.35mA/ppm) and lowest detection limit (0.13ppm) to phenol substrate.

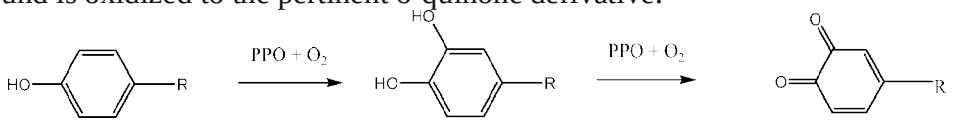
Keywords: Phenolic compounds, biosensor**,** carbon paste, polyphenol oxidaze, crude tissue, Stare case Voltametry (SCV).

Introduction

Phenolic compounds often exist in the wastewaters of many industries [1]. Phenol and related compounds are used extensively in industry in the manufacture of a large variety of aromatic compounds including rubber, fertilizer, paints, drug preparations, petroleum, and agricultural industries. Phenol is reported to be carcinogenic and exposure to phenol results in several symptoms such as convulsions, dizziness and irregular respiration [6]. Some typical phenols as atmospheric pollutants are phenol, o-cresol, m-cresol, and p-cresol; however, phenols are noted more as water pollutants than as air pollutants [1-6]. Many of them are very toxic, showing adverse effects on animal and plants [1]. High levels of phenols have detrimental effects on animal health. Prolonged oral or subcutaneous exposure causes damage to the lungs, liver, kidney and genitourinary tract [1]. In the food industry, phenols are of interest because they are essential compounds of fruit juices, beer, and wines [7].
Since many phenolic compounds can cause bad taste and undesirable odor and are

highly toxic and hazardous to human health [7], their analysis at low concentrations is very important[1.6,7]. 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, biosensors could be a cheap and easy alternative, getting increasing attention in the literature [3]. Electrochemical methods based on enzymes have been widely used for the measuring of phenolic compounds because of the advantages related with good selectivity, long-term stability and potential for miniaturization and automation [4]. The enzyme seems to be of almost universal distribution in animals, plants, fungi and bacteria. Phenol biosensors with whole microorganisms or vegetable tissues are inexpensive and easily produced, but generally exhibit poor selectivity. Compared to free enzyme in solution, the immobilized enzyme is more stable and resistant to various environmental changes [4].

Polyphenol oxidase (PPO) is a bifunctional enzyme responsible for the formation of the natural macromolecule pigment melanin in different species [20]. In its first reaction, monooxygenase activity, PPO catalyze hydoxylates a phenolic substrate at the ortho-position to the hydroxyl group. In the second reaction, oxidase activity, the o-dihydroxy compound is oxidized to the pertinent o-quinone derivative.



Some authors have reported immobilization via entrapment of redox proteins or enzymes, including tyrosinase in membranes consisting of poly(vinyl pyridine), poly(vinyl imiazol), poly(acrylic acid) or poly (allyl amine) [2, 3]. Tyrosinase, a monophenol mono-oxygenase enzyme (PPO enzyme), which catalyzes the oxidation of the phenol group to o-quinone [4] is commonly used in the detection of phenolic compounds.

Immobilization of gold nanoparticles together with redox enzymes for electrochemical biosensors construction provides several advantages as far as biosensor response is concerned.

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 use of plant or mammalian tissues provides numerous advantages for the fabrication of biosensors [11]. 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. However, response times are generally longer because the target substrate must diffuse through the cell membrane before reaching the biocatalyst. In comparison to the isolated enzyme, which can lose its activity during isolation or immobilisation if the process leads to the damage of the active centre, plant tissues provide a novel and cost effective approach to the construction of biosensors [12]. CPE modified with different kind of tissue (as a source of PPO enzyme) was used as the

working electrode in cyclic voltammetry experiments aimed to miniaturization of working potential and quantification of phenolic compounds [9].

Experiment

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 and used without any further purification. Phosphate buffer solution was prepared by mixing suitable amounts of 0.1 M $K_2HPO4 * 3H_2O$ and KH_2PO4, K_2HPO4, KCl was obtained from Merck. Stock solutions of phenol, catechol, hydroquinone, p- Cresol, m-Cresol, 4- chlorophenol, 3-nitrophenol, 4- nitrophenol, 3-nitrophenol were prepared by dissolving the right amount of pure substance in distilled water. The components of the working electrode material were carbon powder prepared through mechanically grinding the pencil leads, the liquid paraffin (Merck product) and banana, apple, mushroom, pear and potato crude tissue (source of polyphenol oxidase enzyme). The mushrooms, potato, banana, pears, apple were purchased from a local market as fresh and culture fruits and vegetables.
Stare Case Voltammetry (SCV) were carried out using Electrochemical analyzer (MEC-12B) using a three electrode system. Home made working electrode was a carbon paste biosensor modified with banana, apple, mushroom, pear and potato crude tissue (source of polyphenol oxidase enzyme), Ag/AgCl reference electrode and a platinum wire as auxiliary electrode . The voltammograms were recorded from $E_i = 0.0V$ to $E_f = 0.8V$, 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. Cyclic voltammetric measurements were conducted at room temperature.

Biosensors construction

To prepare the electrodes material the enzyme crude tissue of different plant (banana, apple, pear, potato, mushroom), the graphite powder, and paraffin were mix together and homogenized. First of all, mix the graphite powder (prepared by grinding mechanically the pencil leads) with the paraffin for 20 min, after that, add the peel of plant tissue, 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 1mL plastic syringe which contains a copper wire to obtain the external electric contact, fig.2 a. The surface of the working electrode was smoothed using a glass surface before the measurements. Unmodified carbon paste electrode was prepared in the similar way without using biological material.
In figure 1 are shown the steps of the preparation of electrode material, construction of the working electrode, electrochemical cell and measurement.

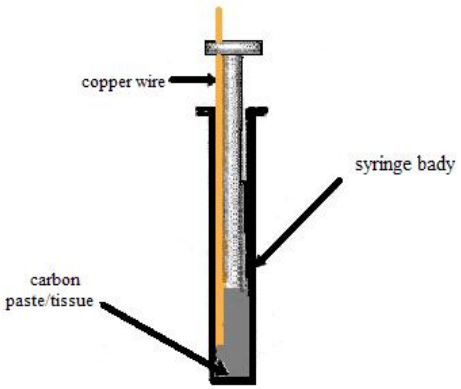


Figure 1 a, Construction of biosensor for phenolic compounds

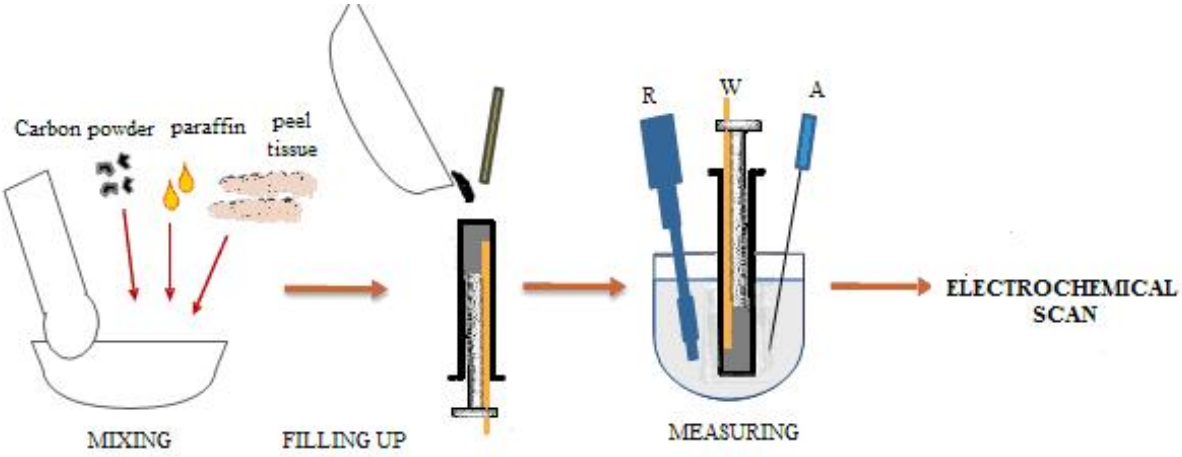


Figure 1 b. Scheme of designed modified biosensors; mixing, connection and electrochemical scan with SCV technique.

Results and discussion

Effect of enzyme as modified component in carbon paste.

In this work the response of a carbon paste electrode modified with banana tissue (CPEB), as an enzymatic source of PPO, was evaluated using cyclic voltammetry (SCV) to test its response to phenolic compounds. The immobilized polyphenoloxidase in the presence of molecular oxygen can catalyses the hydroxylation and oxidation of mono-phenols to o-quinones (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity) [12].

The effect of PPO enzyme incorporated in Carbon paste electrode (CPE), was tested by comparing the voltammograms obtained in the same conditions by both electrodes (unmodified and modified). Figure 2 shows cyclic voltammograms recorded with unmodified

electrode (CPE) and modified electrode (CPEB) in different phenol concentrations, in phosphate buffer pH 7 and scan rate 50 mV/s.

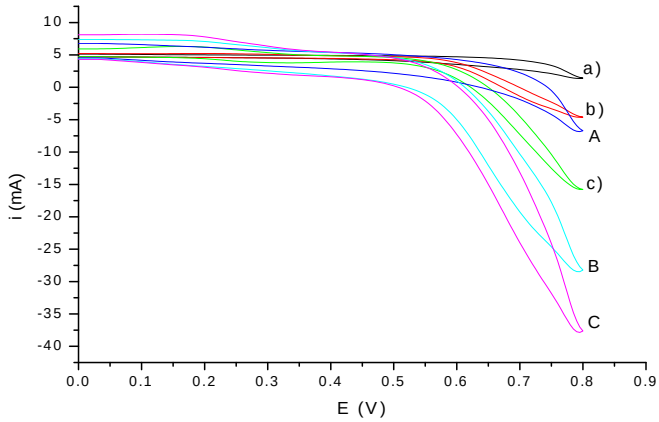


Figure 2. Cyclic voltammograms in 0.1 M phosphate buffer pH 7, Scan rate = 50 mV/s.
using **CPE** (a) buffer, (b) 3.3ppm phenol, (c) 6.6 ppm phenol ;
using **CPEB** (A)buffer, (B)3.3ppm phenol, (C)6.6 ppm phenol.

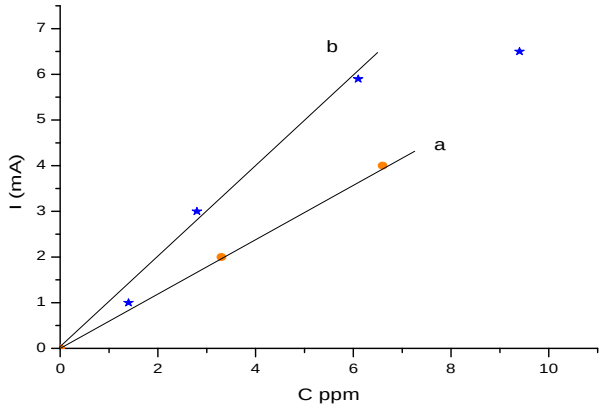


Figure 3. Calibration curves of unmodified (a) and modified electrode (b);

In fig.3 are shown calibration graphs obtained by cyclic voltammograms using unmodified (a) and modified (b) electrodes. It is noticed that up to 7 ppm the sensitivity is higher in the case of modified electrode (S=0.99 mA/ppm) compared with the sensitivity of unmodified (S=0.60mA/ppm). In the higher concentration the behavior of biosensor does not remain linear. Meanwhile both calibration curves have good correlation coefficients R=0.9902 and R=0.9999 respectively for modified and unmodified sensor.

Optimization of biosensor composition

Table 1 summarizes the range over each variable was studied regarding the component amount in the carbon paste and several experimental conditions as well.

The components ratio in the performance of the working electrode material was studied. Using a fixed amount of graphite powder 1.000 g in each paste the amount of paraffin and banana peel was varied. There are experimented electrode material with the amount of paraffin 320, 300, 270, 250 µl and amount of banana peel 0.1, 0.15, 0.2, 0.3g. In each case the response of the biosensor was recorded in 3.3 ppm phenol varying pH and scan rate as are shown in tab 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 was found and optimal experimental conditions were choosen. The modified carbon paste prepared by homogenization of 300 µl paraffin, 0.1g banana peel and 1 g graphite powder resulted with the best analytical performance (sensitivity 0.99mA/ppm R=0.9902) for phenol determination. The highest signal was found at pH 7 using scan rate of 50 mV /s. 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.

Table 1. Optimization of composite biosensor parameters

Parameters	Range studied	Optimal value
Parffin (µl)	250-320	300
Banana tissue (gr)	0.1-0.3	0.1
pH	4-9	7.0
Scan rate / (mV /s)	50-200	50
Potential range / V (CV for phenol)	0.0 to 0.8	0.7

The response of Carbon Paste Electrode modified with bananas tissue (CPEB) in different substrates.

The electrochemical behavior of modified carbon paste electrode with bananas in three different phenolic compounds (phenol, hydroquinone, 3-nitrophenol), were studied. The background current (buffer solution pH=7) was firstly recorded and then the standard solutions of the studied phenolic compounds were introduced into the cell. Based on the recorded voltammograms the catodic current is appiered at 0.3 V in the case of hydroquinone, 0.5V for 3- nitrophenol and at 0.6V for phenol.

The sensor performance to those phenolic compounds is evaluated based on calibration plots (fig.4).

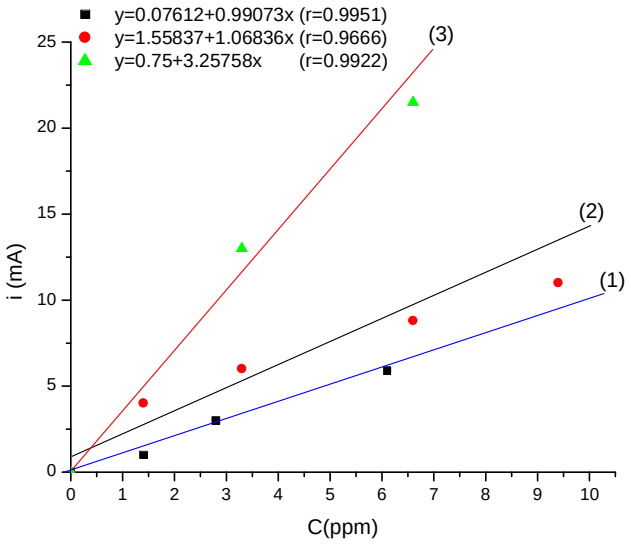


Figure 4. Calibration curves for (1) phenol (2) 3- nitrophenol and (3) hydroquinone using modified carbon paste with banana tissue.

Obviously it is shown that the calibration curves obtained for the studied phenolic compounds vary in sensitivities. The sensitivitiy calculated in the linear range decreases in this order: hydroquinone>3-nitrophenol>phenol.

Table 2 shows the relative response relationship of the composite biosensor (CPEB) to hydroquinone, 3-nitrophenol and phenol. The electrode was more sensitive to hydroquinone (100%), followed by 3-nitrophenol (33%) and phenol (25%). The different responses between those phenolic compounds are due to the different substituent groups and their position. Therefore, higher signals were obtained to ï OH groups inï *para* position once they confer a higher reactivity characteristic to the compounds.

Table 2 . Relative response for different phenolic compounds of CPEB

Phenolic compound	Relative response (%)	Sensitivity mA/ppm	R
Hydroquinone	100	3.26	0.9922
3-nitrophenol	33	1.07	0.9666
Phenol	30	0.99	0.9951

The response of Carbon Paste Electrode modified with different kind of tissue

In this work the PPO based biosensors were prepared using five kind of crude tissues: plant of banana, apple, mushroom, potato and pear. To test the biosensors response the cyclic voltammograms was recorded in phosphate buffer 0.1M pH 7 and scan rate 50 mV/s using phenol as analyte. Table 3 summarizes analytical properties of each biosensor

prepared using different enzyme source: sensitivity (defined by the slope of the linear part), detection limits and standard deviations.

Table 3. Analytical parameters of biosensors obtained prepared using different kind of tissue incorporated at carbon paste electrode.

Enzyme source	R^2 (n=3)	Sensitivity (mA/ppm)	Detection limit (ppm)	SD
1.Unmodified (CPE)	0.9999	0.60	8.88	2.22
2. Banana (CPEB)	0.9951	0.99	1.02	0.31
3.Mushroom (CPEM)	0.9995	0.68	2.12	0.69
4. Apple (CPEA)	0.9523	1.18	4.34	1.32
5. Potato (CPEPo)	0.9059	0.92	3.55	1.50
6. Pear (CPEPe)	0.9643	0.95	2.9	0.91

As it can be seen in table 3 the sensitivity increased from unmodified electrode (S=0.60mA/ppm) to electrode modified with apple tissue (1.18 mA/ppm). The sensitivity can be realted to the level of enzyme activity in those different plant. The highest sensitivity was obtained when apple modification was used followed by the biosensor modified with banana what is previously reported [18].

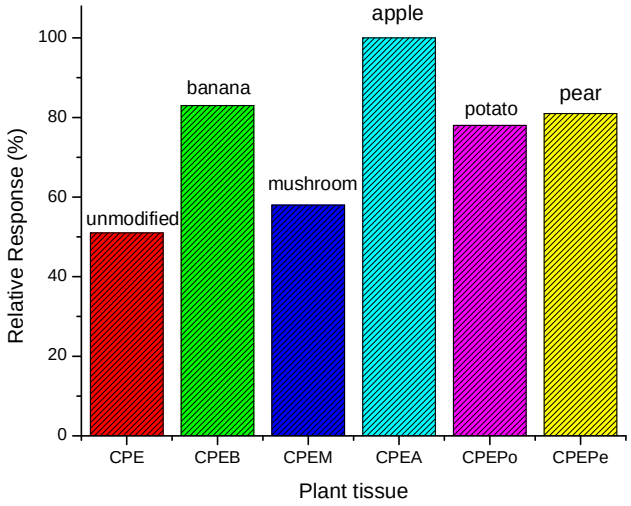


Fig.5. Relative response (%) of biosensor obtained in phenol substrate.The parameter was calculated; considering the response of CPEA 100%.

In fig. 5 is shown a diagram where are compared the response of different modified biosensors in phenol substrate in the same experimental conditions. The parameter is calculated considering the response of CPEA 100%. Modification based on cruide tissue of patato and pear fruit resulted very similar in sensitivity and limit of detection as well.

The response of (CPEA) biosensor to different phenolic compounds

The response of the CPEA biosensor to phenol, 4- clorphenol, hydroquinone, catechol, p-cresol, m- cresol, 3 aminophenol, 3-nitrophenol, 4- nitrophenol was investigated. The SCV technique in phosphate buffer 0.1M , p-H=7, and E=0.0 - 0.8V was used.
The CPEA biosensor had no response towards *m*-cresol, 3- aminophenol, 3- nitrophenol, 4-nitrophenol compounds. It was previously reported that the phenolic compounds with electron-donor substituents in an *ortho and meta*-position gave no response [14].
In Table 4 are summarizes the characteristics of the calibration plots of the studied phenol derivatives, as well as the sensitivity, detection limit and standard deviation.

Table 4. Analytical characteristics of CPEA in various phenolic compounds.

phenolic compound	sensitivity mA/ppm	R	Detection limit (ppm)	SD	Relative response %
4-Chlorophenol	1.35	0.9988	0.82	0.22	100
Catechol	1.17	0.9763	1.99	0.91	86
Hydroquinone	0.59	0.9462	3.69	0.88	61
p-Cresol	0.48	0.9697	1.61	0.42	31
Phenol	0.23	0.9991	0.13	0.04	18
m-Cresol	no response				-
3- Aminophenol	no response				-
4-Aminophenol	no response				-
3- nitrophenol	no response				-
4-nitrophenol	no response				-

The detection limit ranged between 0.82 and 1.99 ppm for the tested phenol derivatives. The different sensitivities varied between 0.23- 1.35 mA /ppm for the tested phenolics can be related to the formation of *o*-quinones during the enzymatic reaction [19]. The maximum sensitivity was found to be 1.35 mA /ppm for 4-clorphenol. The difference in sensitivity between each mono-phenolic compound might be depended on the hydrophobic characteristics of the immobilisation matrix (carbon paste) [25].
The CPEA biosensor showed high sensitivity for catechol (S=1.17mA/ppm). This can be related with the presence of ï OH group of catechol in orto position which enhances oxidation of the o-diphenol to quinone by PPO enzyme.
In Table 4 are shown the relative response relationship of the CPEA biosensor in the same experimental conditions in the solutions of catechol, hydroquinone, 4-chlorophenol, phenol, p-Cresol, m-cresol, 4-nitrophenol, 3-nitrophenol, 3-aminophenol and 4-aminophenol. The values are calculated considering the response of CPEA in 4-chlorphenol 100%. The different responses between those phenolic compounds are due to the different substituent groups and their position. Therefore, good signals were obtained to ï OH groups in ï *ortho* position that confer a higher reactivity characteristic to the compounds.

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