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Determination of phenols in underground waters using carbon paste biosensor modified with banana crude tissue

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PHENOLS

Groundwater:

high quantity of inorganic and organic compounds

One of the aims of the water analysis is to obtain better knowledge concerning:

- the water quality
- residence times in the aquifer
- Age
- recharge areas
- flow paths
- and a potential or prohibitive use due to human pollution problems [1].

Phenolic compounds, (chlorophenols and nitro phenols)

- ✓ are toxic
- ✓ highly resistant to biological degradation
- ✓ persistent in the environment.

(EPA) and the European Union have included these phenolic compounds in their list of primary pollutants [2-3]

Therefore, the reliable and effective determination and disposal of phenolic compounds are very important and has long been of interest [3]

Carbon Paste Electrodes

Widely applicable in electrochemical studies due to

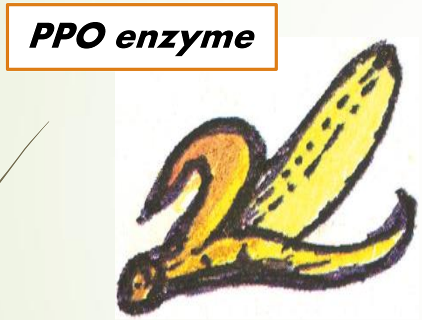
- **feasibility to incorporate different substances during the paste preparation,**
- **their low background current (compared to solid graphite or noble metal electrodes),**
- **simple renewal of their surface,**
- **easy preparation,**

low cost,

- We fabricated an electrochemical biosensor modified with crude tissue of banana, source of PPO enzyme for rapid, sensitive and selective determination of phenols.

Crude Tissue as Sources of Enzyme for Biosensor Preparation

Many fruits and vegetables are rich in enzymes and can be used in biosensors construction



Advantages:

- *the enzyme is in its natural environment*
- *may be used without preparatory work*
- *higher enzyme activity compared with biosensors modified with purified enzyme*
- *cofactors may be already present*
- *lower cost*
- *simplicity of the biosensor construction*

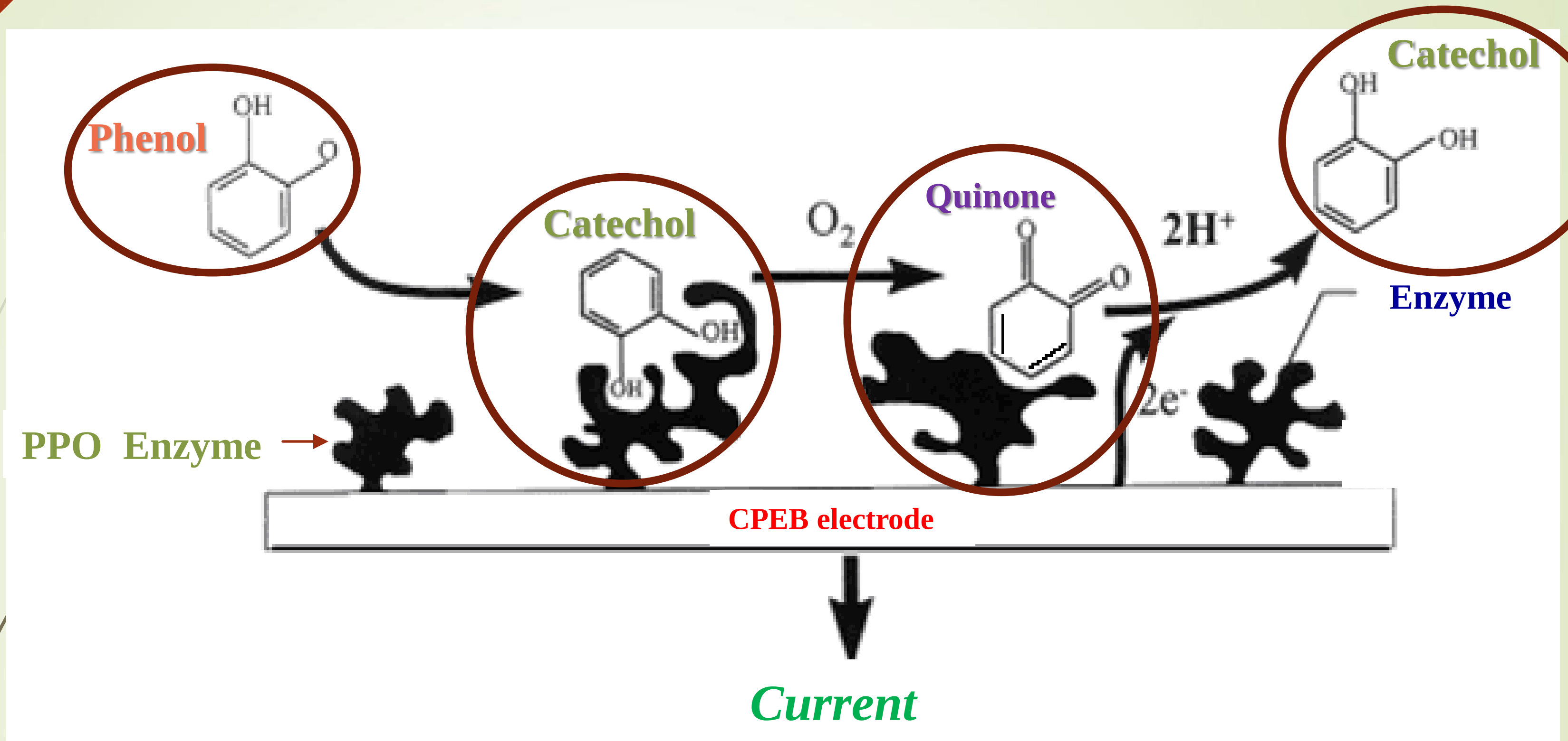
Disadvantages:

- ***The tissue contains more than one enzyme***

(problem with selectivity)

Polyphenol oxidase (PPO)

The scheme of the enzymatic and electrochemical reactions at biosensor surface.



➨Polyphenoloxidase enzyme catalyses' the oxidation of o-diphenols to o-quinones by consumption of molecular oxygen.

➨These quinones are electrochemically reduced at a low potential and the measured current is proportional to the phenol concentration [8, 9].

Experimental

- **Equipment**

- **R:** Reference electrode (Ag/AgCl)
- **W:** Working electrode
- **A:** Auxiliary electrode

- **Electrochemical cell**
- **Analyzer (MEC-12B)**
- **Computer**
- **Software**

Voltammetric techniques

- The voltametric measurements were performed with Electrochemical analyzer (MEC-12B) using a three electrode system.

- The electrochemical cell was a three-electrode cell:

  1. The working electrode was a homemade carbon paste biosensor modified with banana crude tissue
  2. Ag/AgCl reference electrode
  3. A platinum wire as auxiliary electrode.

- The cyclic voltamogramms were recorded from -0.4 V to 1.0 V, in 0.1M buffer solution (pH=7) and amplitude wave 30 mV.

✓In each case the background voltammogram was firstly recorded and then the additions of phenol standard solution were introduced into the cell.

Biosensors construction

✓ The modified carbon paste sensors were prepared by mixing firstly the graphite powder and paraffin and then the modifier (banana tissue) was added and mix for at least 20 min to obtain a homogenous paste. The paste was stored at 4°C overnight.

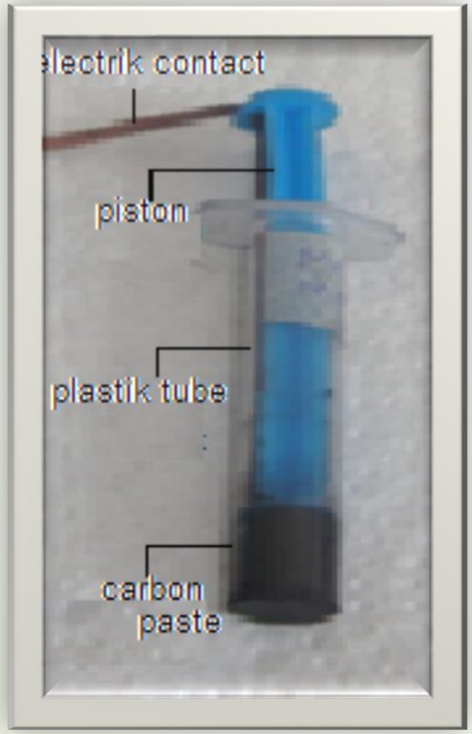
✓ Unmodified carbon paste electrode was prepared in the similar way without using biological material.

❑ Sample Preparation and Measurement

Underground water samples were stored in a refrigerator immediately after collection. Firstly the water samples were cleaning by undergoing a distillation process to minimize interference during electrochemical determination of phenol. After collecting 500 ml of distillate, 5 ml samples was added into electrochemical cell containing 15 ml of 0.1M phosphate buffer (pH = 7) and analyzed using CPEB biosensor after successive additions of the standard phenols solution. Studied phenols are very volatile and the samples were analyzed within a short time and for how long are used they are stored in the refrigerator.

Home made Biosensor

- A portion of paste was packed into the tip of a plastic syringe (geometrical area of 0.5 cm^2)



- A copper wire was used for the external electric contact.

- The surface of the working electrode was smoothed using a clean glass surface before the measurements.

Results and discussion

Optimization of the biosensor response

Parameter	Optimized Value
• Electrode composition	1.0 g of graphite powder+ 0.1 g banana tissue + 250 µl of paraffin
• Supporting Electrolyte	(5.795g $K_2HPO_4x3H_2O$, 2.27 g KH_2PO_4 and 2.932 g KCl) 0.1M
• pH	7.0
• Amplitude	30 mV
• Step potential	10 mV
• E_{init}	-0.4 V
• E_{fin}	+1.0 V
• Scan rate	50 mV/s

- Based on the performance of the biosensor the optimal ratio between components of the electrode material was found to be 250 µl paraffin and 0.100g banana peel homogenized with 1.000 g graphite powder.
- The optimal pH using optimal electrode material was found at pH 7.
- In this study, a scan rate of 50 mV /s was chosen to achieve a cathodic peak current at 0.35 V.

Catalytic Effect of PPO Enzyme

The CPE electrode produced a relatively weak cathodic reduction peak, whereas CPEB produced a high reduction peak in the same concentration of hydroquinone

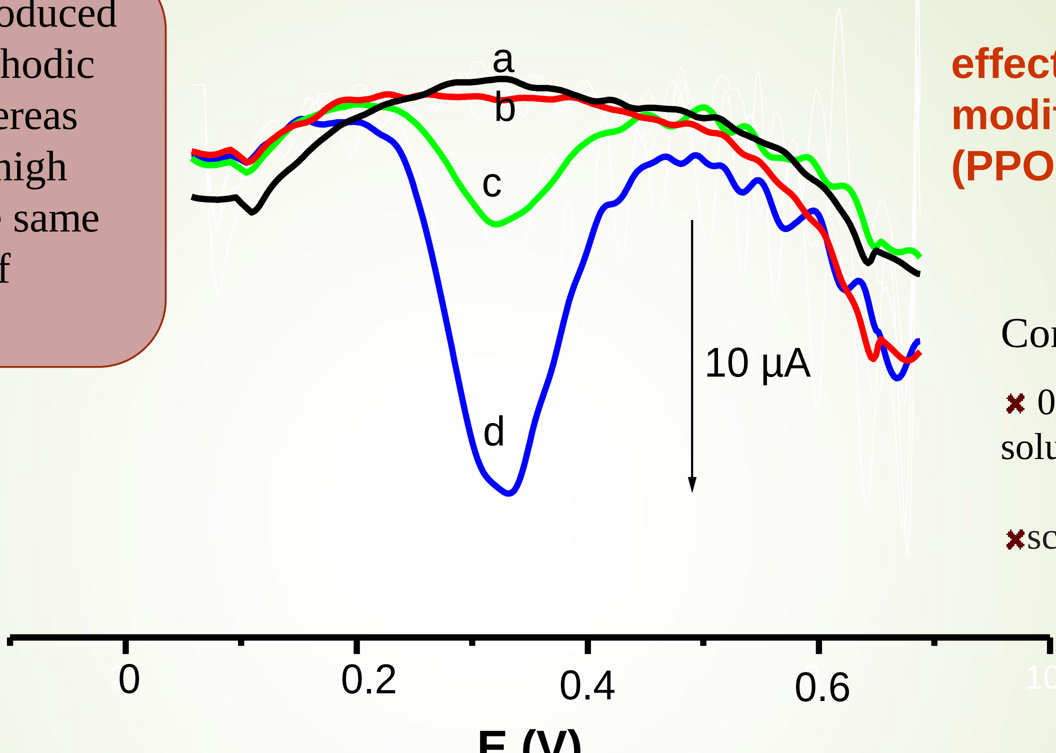


Figure 1. Voltammograms of CPE (b), CPEB (a) in the buffer solution; in 2.99 x 10^{-5} mol/L hydroquinone CPE (c), CPEB (d);

effect of biological modifier (PPO-enzyme) ?

Conditions:

- 0.1 mol/L buffer solution pH=7
- scan rate 50 mV/s.

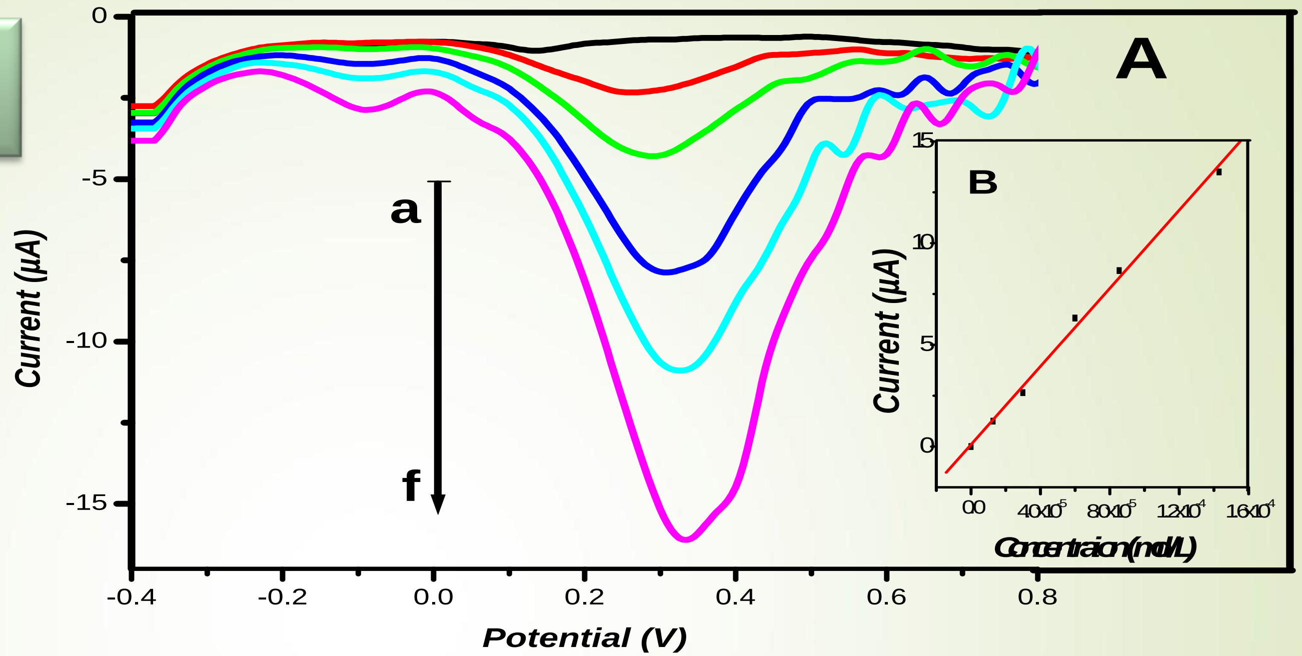
With the catalysis of PPO, oxygen can oxidize hydroquinone into *p*-benzoquinone and *p*-benzoquinone can produce reduction current on the electrode. A cathodic potential scan detected the reduction current of *p*-benzoquinone at potential 0.35 V (vs. Ag/AgCl).

Analytical Performance of Biosensor

✓Sensitivity was found 1.75 x 10^{-5} µA/mol/L

✓correlation coefficient of 0.9981

✓limit of detection (S/N = 3) of 1.9 x 10^{-6} mol/L



Inset A. Square Wave Voltammograms responses of the CPEB electrode in 0.1M phosphate buffer solution with increasing hydroquinone concentration of 1.48 x 10^{-5}, 2.99 x 10^{-5}, 5.99 x 10^{-5}, 8.54 x 10^{-5}, 16.7 x 10^{-5} mol/L from a to f, pH 7, amplitude 30 mV and step potential 10 mV. *Inset B*. Linear regression curve of reduction peak current and concentration of hydroquinone

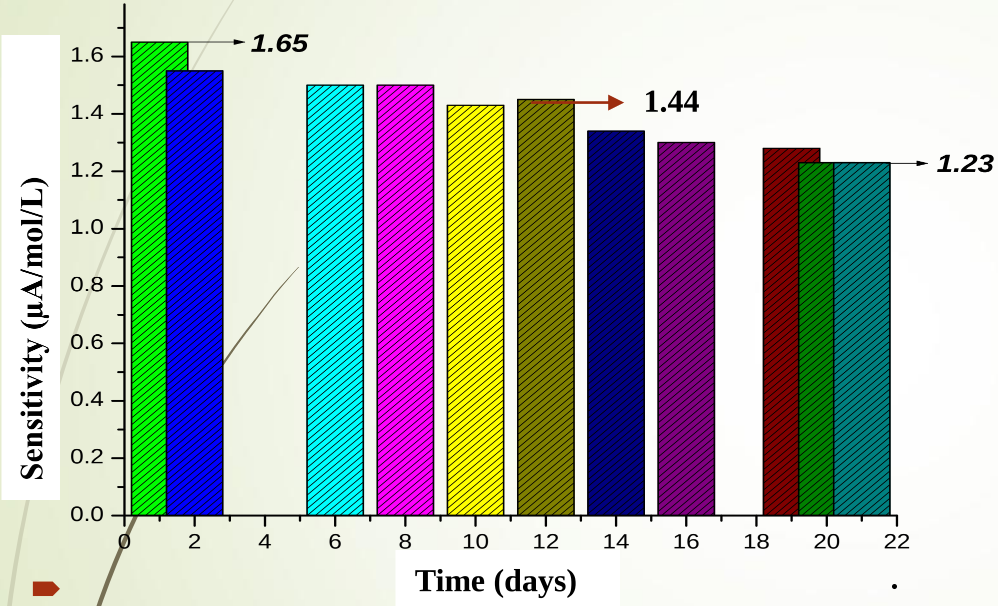
The cathodic peak currents obtained in the square wave voltammetry were proportional the hydroquinone concentrations of 1.48 to 16.7 x 10^{-5} mol/L

Thus CPEB has good electrocatalytic activity and adsorption capacity towards high concentrations of hydroquinone, so it could be used to detect high concentrations of hydroquinone.

Reproducibility, repeatability and stability of the CPE-B

➨Reproducibility, Repeatability of the signal obtained in 1.48 x 10^{-5} mol/L hydroquinone resulted:

The stability of CPEB biosensor :
it was studied by measuring its response to hydroquinone in phosphate buffer solution (pH=7), in different days within 3 weeks.



➨ It was observed that after 12 days the current obtained in the same experimental conditions and sensitivity resulted lower (from 1.65 to 1.44 μA/mol/L) and after 22 days 1.23 μA/mol/L .

✓Good Reproducibility (n=3) (RSD 3.6%)

✓ and Repeatability (n=5) (RSD 3.2 %)

✓ The response of biosensor decrease after 22 days

These results indicate that PPO enzyme in banana fruit tissue was denatured in presence of molecular oxygen, as a result of successive reactions that occur during the process of decomposition of phenolic compound in this plant tissue [16, 17]. Therefore the CPEB biosensor should be prepared freshly for analytic determination and can be stored up to one week.

REAL SAMPLE ANALYSIS

- The applicability of the propose voltammetric biosensor was checked with determination of phenols (hydroquinone, 4-clorphenol, p-cresol, m-cresol, phenol) in real samples such as underground waters

Standard addition method and SWV technique

- Experimental results obtained by electrochemical detection of phenols in underground water sample (n=3)

Underground water samples Amount (mol/L)			
4-Clorophenol	p-Cresol	m-Cresol	Phenol
$1.9 \times 10^{-5} \pm 0.02$ mol/L	$2.2 \times 10^{-5} \pm 0.1$ mol/L	$1.8 \times 10^{-5} \pm 0.02$ mol/L	$3.6 \times 10^{-5} \pm 0.1$ mol/L

No hydroquinone signal was detected in sample, it might be because there was no hydroquinone in the sample or the hydroquinone concentration was below the detection limit of the biosensor

Quality control of analytical results based on recovery of standard addition

✓good selectivity

Pollutant	Amount added (mol/L)	Found[a] (mol/L)	RSD[b] (%)	Recovery (%)
Hydroquinone	4.99×10^{-6}	4.90×10^{-6}	2.77	98.2
	9.99×10^{-6}	9.18×10^{-6}	2.02	91.8
4-Chlorophenol	4.98×10^{-6}	4.85×10^{-6}	8.73	97.4
	9.96×10^{-6}	10.03×10^{-6}	8.40	100.7
m-Cresol	4.99×10^{-6}	4.80×10^{-6}	1.96	96.2
	9.98×10^{-6}	9.70×10^{-6}	4.70	97.2
p-Cresol	4.99×10^{-6}	4.90×10^{-6}	4.00	98.2
	9.98×10^{-6}	10.07×10^{-6}	8.25	100.9

[a]Average value of three measurements. [b] Relative standard deviation for the proposed method (n = 3).

The results clearly demonstrate and confirm the capability of the modified electrode (CPEB) for the voltammetric determination of phenol, 4-clorphenol, p-cresol, m-cresol with good selectivity and good reproducibility. Our electrochemical biosensor is a reliable and effective for determinations of phenols in real samples

Conclusions

- By modifying a carbon paste electrode with banana crude tissue, we successfully prepared an electrochemical biosensor that could be used to detect the concentration of phenols in underground water samples

- Because of the catalytic effect of PPO enzyme on the surface of the electrode could realize good electrocatalytic activity and generate an electrochemical response to phenols. The reduction peak current increased for the electrode containing biological material compared with that of a bare one.

- The biosensor accurately detected the concentration of phenols in actual underground water samples, and gave satisfactory recoveries of p-cresol, hydroquinone, m-cresol and 4-chlorophenol.

- Our results indicate that CPEB electrode can be used to rapid and sensitive detection of the concentration of phenols in water samples with good reproducibility and stability.

Thank You for Your Attention