

## Quantitative determination of phenol in natural decayed leaves using procaine modified carbon paste electrode surface by cyclic voltammetry

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### ABSTRACT

In this research, the amount of phenol in decayed leaves was quantified in aqueous medium by using procaine hydrochloride modified carbon paste electrode. Working electrode was prepared by using homogeneous mixture of graphite powder and mineral oil (80:20%). The mixture was pressed on the electrode surface thus a glassy form was obtained. The electrode surface was modified with procaine hydrochloride in 0.1 M tetrabutylammonium tetrafluoroborate using 100 mV s<sup>-1</sup> scanning rate between -1.7 V and -0.5 V with 50 cycles. After the modification process, the electron transfer becomes easier and faster. For the calibration curve, a series of standard phenol solution between 1.0 × 10<sup>-3</sup> M and 1.0 × 10<sup>-8</sup> M was prepared using Britton–Robinson buffer solution at pH 12. The prepared standard solutions were used for the chemical binding of phenol to the modified electrode surface between 0.0 V and +0.9 V potential range using 100 mV s<sup>-1</sup> scanning rate with 10 cycles. Calibration curve was obtained by plotting concentrations versus peak currents of standard phenol solutions. By using this calibration method, the amount of phenol was determined as 8.33 × 10<sup>-5</sup> M in natural decayed leaves in BR buffer solution at pH 12. Detection limit was obtained as low as 1.0 × 10<sup>-8</sup> M.

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### 1. Introduction

Electrochemical methods are based on the direct oxidation or reduction of substrate onto an electrode surface. Electrode reactions are very suitable for analytical applications due to their requirements of high potential. Moreover, these surfaces can be modified by a reductive substrate for analytical applications. Recently, the application of inorganic modified electrodes has increased [1–5]. Anodes, suitable for the active and high positive potentials are important for the electro-oxidation of organic pollutants. However, oxygen is released at higher potential which interferes obtaining good results [6–8].

In the electro-oxidation, hydroxylation and hydrogen evolution take place which is a favored situation due to hydroxy adsorption [9]. Radical forms are pacified on the electrode surface through polymerization [10]. The studies of phenolic compounds and total mineral concentration are difficult and different from each other [11].

The determination of phenolic compounds and their toxic effects has a very important role in production process. Besides colorimetric and UV-spectrophotometric analysis as standard methods are still widely used. Determination of phenol in environmental samples has triggered the development of new methods [12,13].

Phenols are derived from two main sources: one is naturally, the other is produced in big amounts in different places. Phenol naturally exists in some vegetable, human and animal waste and decomposed organic materials. It is also produced in our body during food intake. Phenol is used as a starting material for the production of phenolic resins. Phenol is used in the cleaning process of bacteria and fungi and for the treatment of sour throat as a paste and solution in medicine due to its disinfectant properties. Phenol today is not only threatening the human health but also is a major source of pollutant, thus, it carries a potential risk for usage. In addition to this, phenol derivatives are important in the aspect of biology (catecholamines, pharmaceutical products etc.) and industry (antioxidant, antimicrobial agents etc.). Phenol naturally exists in environmental science, water and food. For this reason, phenol should be determined quantitatively when the sample is analyzed by sensitive analytical methods [14–20]. Phenol is a pollutant in water, can food, atmosphere and chemical industry [21].

Phenol can be bind onto an electrode surface thus a polymerization process takes places on the modified electrode surface. The binding of phenol on the electrode surface enable us to determine phenol at very low concentrations. Phenol determination is very difficult in natural samples due to its dissolution mechanism in water which is given in Fig. 1. Voltammetric techniques require a small amount of sample volume thus help us to determine phenol and its derivatives.

The reason why we applied this method to the decayed leaves is phenol content is more in decayed leaves than the other samples. When the leaves decay, the phenol content increases. Decayed leaves

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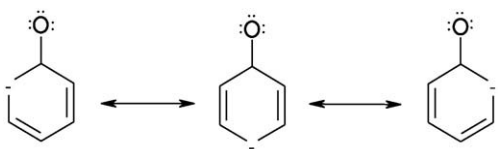


Fig. 1. Behavior of phenoxide ions in aqueous solution.

are generally in contact with water. This could lead to the transfer of phenol to the water thus endangers the human and animal life.

Procaine was first synthesized in 1905. It was created by the German chemist Albert Einhorn (1857–1917) who gave the chemical the trade name Novocaine, from the Latin *Novus* (meaning New) and *caine*, a common ending for alkaloids used as anesthetics. It was introduced into medical use by surgeon Heinrich Braun (1862–1934).

Local anesthetic is a substance that causes loss of sensation only to the area to which it is applied without affecting consciousness. Most local anesthetic molecules have amino-ester or an amino-amide group which are linked to hydrophilic (secondary or tertiary amine) and to hydrophobic group (aromatics) on the other side. Procaine, an ester anesthetic, is used as a local anesthetic agent that gives prolonged relief from pain in medicine and dentistry. It has the advantage of restricted blood vessels.

## 2. Experimental

### 2.1. Reagents

Ultra pure high quality of water having a resistance of 18.3 MΩ cm (Millipore Milli-Q purification system) was used in preparations of aqueous solutions, cleaning of the glassware and polishing the electrodes. All chemicals were of the highest purity available from Merck, Fluka, Riedel or Sigma-Aldrich chemical companies and so no further purification was performed. In all experiments, the solutions and the electrodes were kept in acetonitrile when they were not in use. All the experiment solutions were prepared at 1 mM concentration used in surface modification. Solutions were thoroughly deoxygenated by purging with purified argon gas (99.99%) for 10 min prior to the electrochemical experiments. Argon blanket was maintained over the solutions to supply an inert atmosphere during voltammetric measurements. All electrochemical experiments were performed at room temperature ( $25 \pm 1^\circ\text{C}$ ). Naturally decayed leaves were prepared in BR buffer solution at pH=12 after being dried in air and grinded to powder.

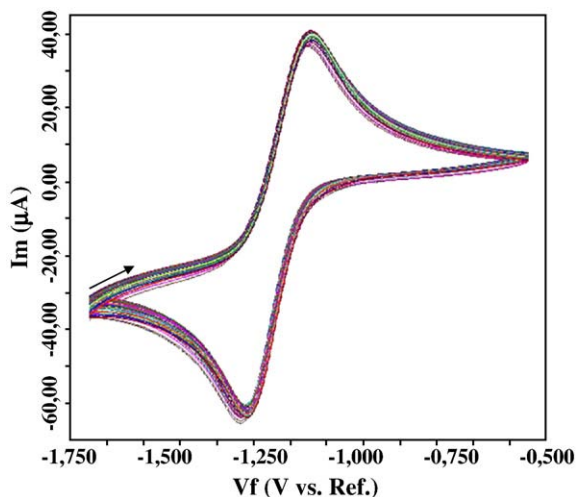


Fig. 2. PR modification voltammogram on CPE surface into  $-1.7\text{ V}$  and  $-0.5\text{ V}$  potential range using  $100\text{ mV s}^{-1}$  scanning rate with 50 cycles.

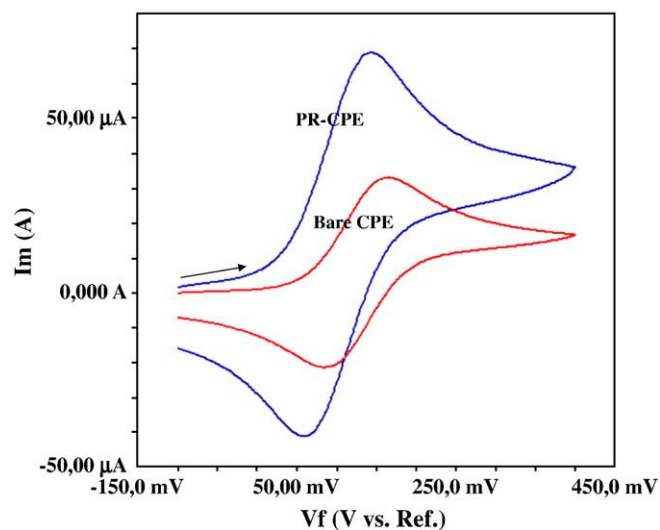


Fig. 3. Overlaying the bare CPE and PR modified CPE voltammograms.

### 2.2. Electrodes and apparatus

All voltammograms were recorded with a voltammetric analyzer GAMRY Reference 600 and GAMRY 750 potentiostat/galvanostat/ZRA with C3 cell stand (Bioanalytical Systems, Inc., BAS in USA). A three-electrode cell was employed incorporating a carbon paste electrode (BAS Model MF-2010, 3 mm diameter) as working electrode,  $\text{Ag}/\text{Ag}^+$  (0.01 M  $\text{AgNO}_3$  in 0.1 M TBATFB) (BAS Model MF-2042) for non-aqueous medium and  $\text{Ag}/\text{AgCl}/3\text{ M KCl}$  (BAS Model MF-2063) for aqueous medium as reference electrodes and a platinum wire (BAS Model MW-1032) as auxiliary electrode. Reference electrodes calibrated to the  $E_{1/2}$  of ferrocene in 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) and ferricyanide in 0.1 M  $\text{H}_2\text{SO}_4$ . The pH of buffer solution was measured with a Denver Instrument digital pH-meter with glass combination electrode.

### 2.3. Preparation of the electrodes

The working carbon paste electrode (CPE) was prepared by mixing 80% (w/w) graphite powder and 20% (w/w) mineral oil. The electrode tip (3 mm diameter, 1 mm depth) was filled with this mixture by pressing to obtain a glassy surface. Prepared carbon paste electrode was

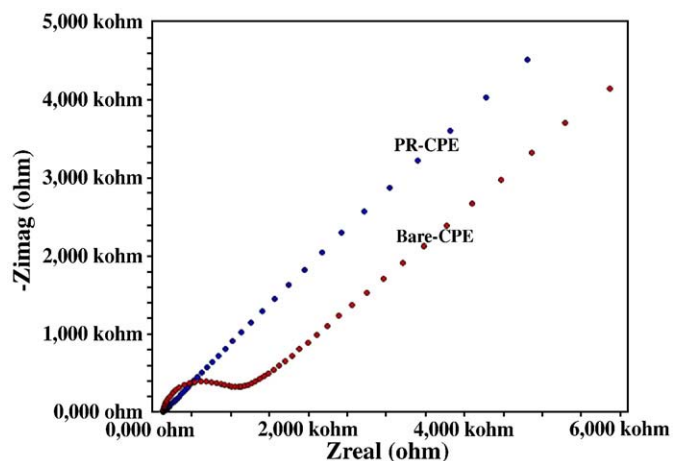


Fig. 4. Overlaying the impedance plots obtained by 1 mM  $\text{Fe}(\text{CN})_6^{3-/4-}$  redox probe in 0.1 M KCl.

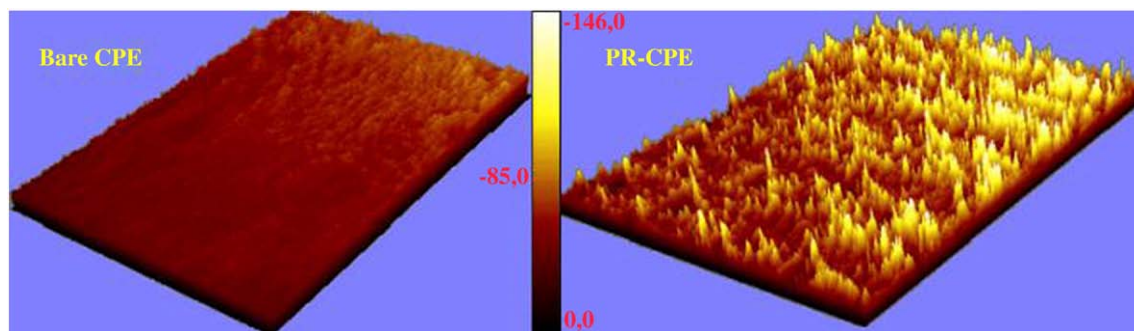


Fig. 5. Surface of bare CPE and PR modified CPE taken by ellipsometry.

polished to a smooth shiny finish by gently rubbing over an ordinary weighing paper [22]. The electrode was stored at +4 °C until use.

### 3. Results and discussion

#### 3.1. Modification and characterization of PR on CPE

To bind phenol electrochemically onto the procaine (PR) modified CPE surface, 1 mM PR solution in 0.1 M TBATFB was used in the  $-1.7$  V and  $-0.5$  V potential range using  $100 \text{ mV s}^{-1}$  scanning rate with 50 cycles [23]. Reaction mechanism for the binding process is shown in Fig. 2. From the ferrocene test by CV and impedance measurement of ferricyanide/ferrocyanide using redox probe, PR modified CPE surface was found to be more active and faster electron transfer took place on this surface after the modification process [24–26].

Solutions were thoroughly deoxygenated by purging with purified argon gas (99.99%) for 10 min prior to the experiments. Argon blanket was maintained over the solutions to provide an inert atmosphere during all voltammetric and spectroscopic measurements.

Surface characterizations after the modification process were carried out by CV in non-aqueous and by Electrochemical Impedance Spectroscopy (EIS) in aqueous medium. In the characterization with CV, 1 mM ferrocene solution in 0.1 M TBATFB was performed in the  $-0.1$  V and  $+0.4$  V potential range using  $100 \text{ mV s}^{-1}$  scanning rate and shown in Fig. 3. The characterization process by EIS, 1 mM ferricyanide/ferrocyanide in 0.1 M KCl (1:1) mixture was used for the impedance measurement using 100,000/0.05 Hz frequency range and shown in Fig. 4.

Ellipsometry was used to measure the thickness of electrode surfaces in three dimensions. The optical thickness of modified surface

was measured at 532 nm wavelength with  $73^\circ$  incident beam. Four-zone auto-nulling procedure technique was used to scan an area having  $50 \mu\text{m} \times 50 \mu\text{m}$  dimensions for the thickness measurement. Graphite, having  $n=3.0841$  (real refractive index) and  $k=1.7820$  (extinction coefficient), was chosen as a model for the carbon paste electrode. These values were measured as  $n=1.46$  and  $k=0$  for the modified electrode surface. From the measurements there is a  $5.41 \pm 0.043$  nm difference in height between bare CPE and PR modified CPE surfaces. This difference proves that CPE surface is modified with PR and it is shown in Fig. 5.

#### 3.2. Determination of optimum pH using BR buffer solution

In the current study, BR buffer solution was used at 2–12 pH range. 1 mM phenol solutions were used to modify the bare CPE and peak currents were recorded and it is shown in Fig. 6. Fig. 7 shows the relation between the peak current values and pH obtained from the first cycle. For all solutions, the modification was performed in 0.0 V and  $+1.5$  V potential range using  $100 \text{ mV s}^{-1}$  scanning rate with 10 cycles. pH 12 was found to be the optimum value for the binding of phenol to the bare CPE and was used throughout the experiments. The obtained voltammogram is shown in Fig. 8.

#### 3.3. Modification and characterization of phenol on PR-CPE

To the PR-CPE surface, phenol binding was performed in BR buffer solution at pH 12 with 1 mM phenol solution in 0.0 V and  $+1.5$  V potential range using  $100 \text{ mV s}^{-1}$  scanning rate with 10 cycles and it is shown in Fig. 9. Surface characterization process for the procaine modified CPE surface was performed using CV in non-aqueous and using EIS in aqueous media and the same conditions were applied for phenol bind surface. In the characterization process done by CV, 1 mM ferrocene (in 0.1 M TBATFB) solution was used in  $-0.1$  V and  $+0.4$  V potential range

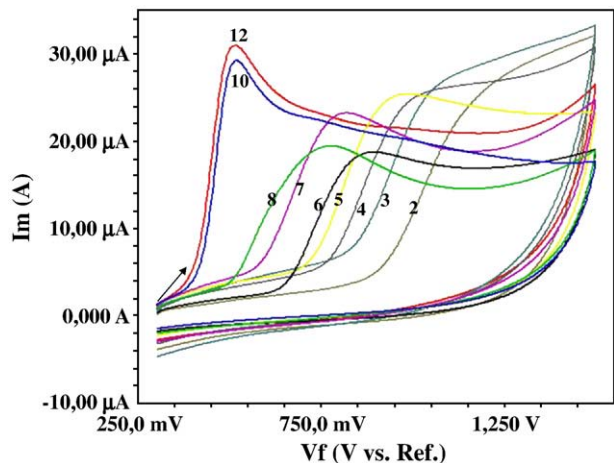


Fig. 6. An overlaying voltammogram for phenol binding to the PR modified CPE surface at different pH 2–12.

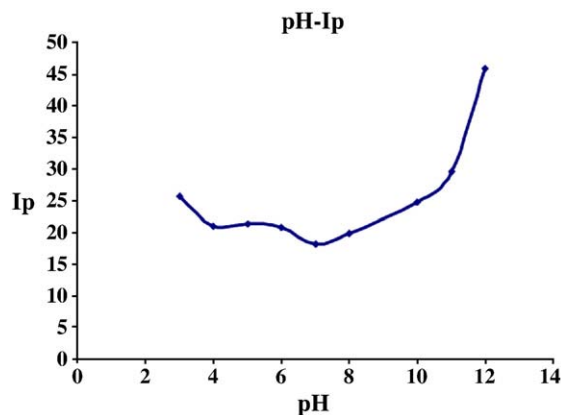


Fig. 7. A plot of peak current was pH obtained from different phenol solutions.

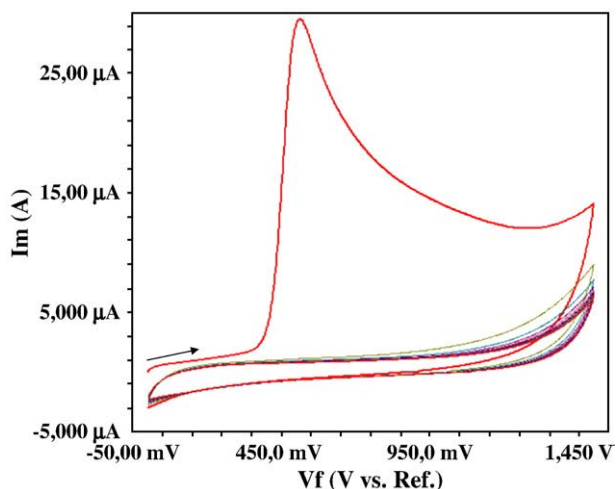


Fig. 8. The phenol modification voltammogram in BR buffer solution at pH 12, 0.0 V and +1.5 V potential range,  $100 \text{ mV s}^{-1}$  scanning rate.

using  $100 \text{ mV s}^{-1}$  scanning rate and it is shown in Fig. 10. As it is seen from the figure, the electron transfer takes place on the bare CPE and PR modified CPE while it does not take place on the phenol bind CPE surface. This proves the binding of phenol to the modified CPE surface. For the characterization process performed by EIS, 1 mM ferricyanide/ferrocyanide (1:1) (in 0.1 M KCl) solution was used to obtain the impedance in 100,000 and 0.05 Hz frequency range and it is shown in Fig. 11. Following the impedance measurement, the simulation was done for the obtained plot to suggest an equivalent circuit model using the calculated parameters  $R_p = 2.25 \times 10^5 \Omega \text{ s}$ ,  $R_u = 236.2 \Omega \text{ s}$ ,  $Y_0 = 1.25 \times 10^{-7} \text{ S}$ ,  $\alpha = 0.810$ . Non-allowed electron transfer of phenyl bind PR-CPE surface was proven not only by ferrocene test but also by EIS due to the enormous amount of resistance against electron transfer. PR modified CPE surface provides twice faster electron transfer than bare CPE surface because the peak current obtained from phenyl bind PR-CPE surface was almost twice the peak current obtained from the phenyl bind bare CPE surface.

Based on the results of the study done by using phenol standard solutions, the following reaction mechanism is suggested for the modification of procaine to the bare CPE surface and for the binding of phenol to the PR modified CPE surface. This is shown in Fig. 12. We suggest that phenol (exists as phenoxide ion) bind to amine surface through N–O covalent bonding.

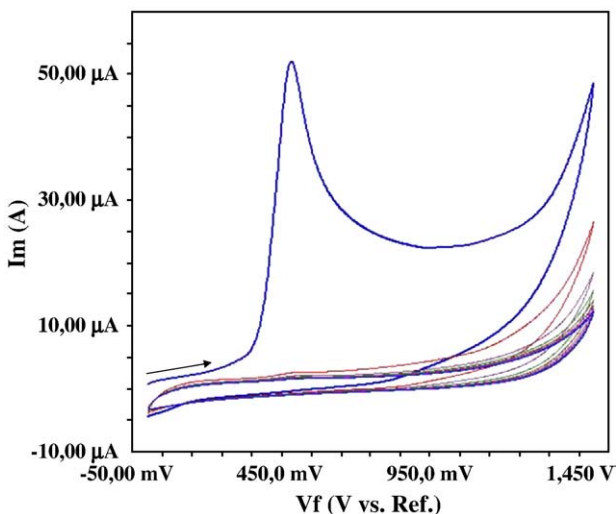


Fig. 9. Voltammogram for phenol binding to the PR modified CPE surface in BR buffer solution at pH = 12.

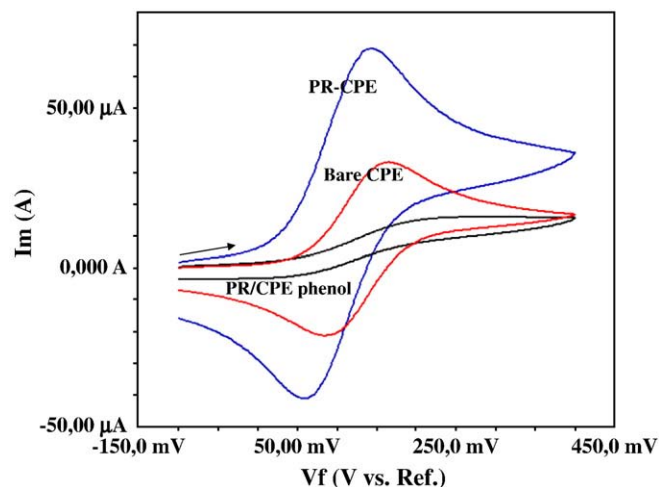


Fig. 10. An overlay voltammograms of ferrocene surface with bare CPE, PR modified CPE and phenyl binded PR modified CPE surface.

### 3.4. Quantitative determination of phenol by PR-CPE

A series of phenol solutions in  $1 \times 10^{-3} \text{ M}$  and  $1 \times 10^{-8} \text{ M}$  range was prepared in optimum conditions. Phenol binds to the PR modified CPE surface for each standard solution. First cycle peak currents obtained from the oxidation peaks, were recorded for all standard solutions. The peak current values were plotted against phenol concentrations and it is shown in Fig. 13. When the phenol concentration is below  $1 \times 10^{-8} \text{ M}$ , no peak is observed. Therefore, this limiting concentration was accepted as detection limit for phenol quantification.

To apply the suggested method to the natural samples, decayed leaves from the trees on roadside were collected in Meram region in Konya. Leaves were dried on a filter paper in a closed room. Dried leaves were grinded to powder. A 5 g of sample was weighed and mixed with 50 mL of BR buffer solution at pH = 12. The mixture was mixed with a magnetic stirrer for 12 hours and then, it was filtered. A small portion of filtered solution was analyzed for its phenol amount. The obtained voltammogram is shown in Fig. 14.

From the plot, peak current value was read  $10.20 \mu\text{A}$ . When it is calculated from the standard curve equation, read peak current value corresponds to the  $8.33 \times 10^{-5} \text{ M}$  phenol concentration.

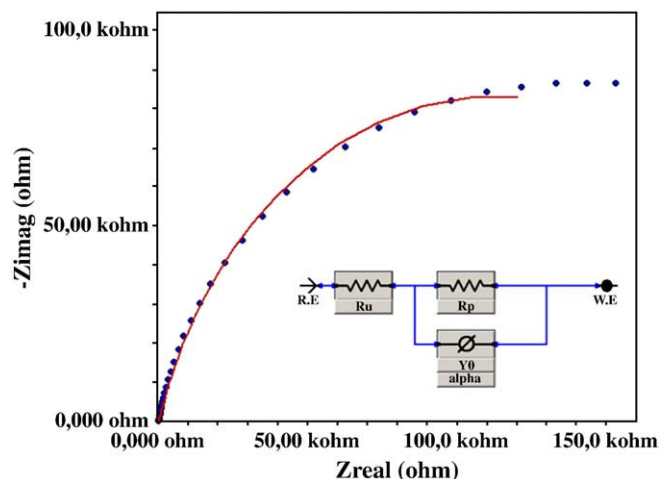


Fig. 11. Impedance simulation of phenol binding PR modified CPE surface.

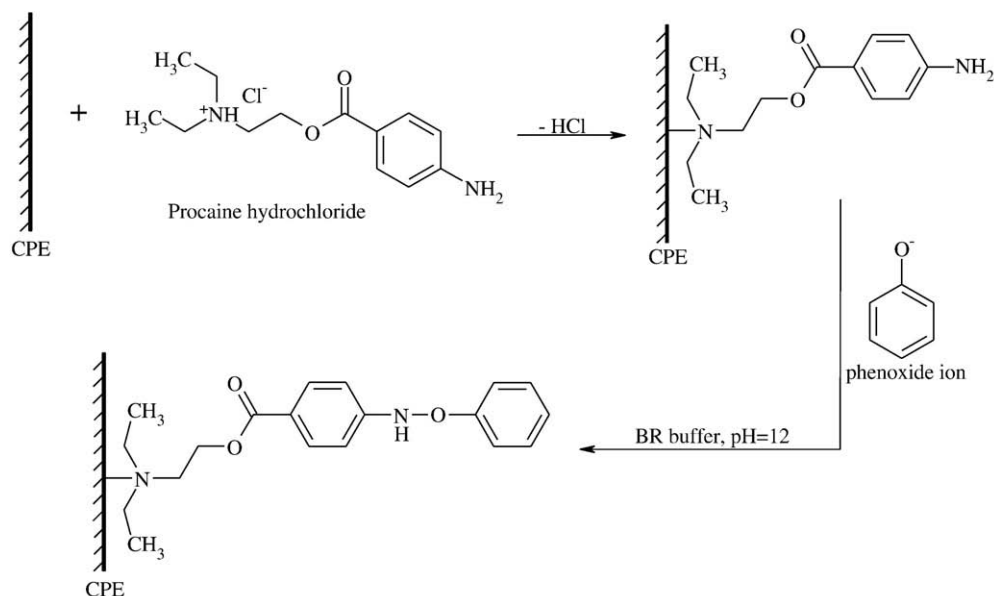


Fig. 12. Reaction mechanism for the procaine binding to CPE surface ( $-1.7$  V and  $-0.5$  V potential range using  $100$  mV s<sup>-1</sup>) and phenol binding to PR modified CPE surface ( $0.0$  V and  $+0.9$  V potential range using  $100$  mV s<sup>-1</sup>).

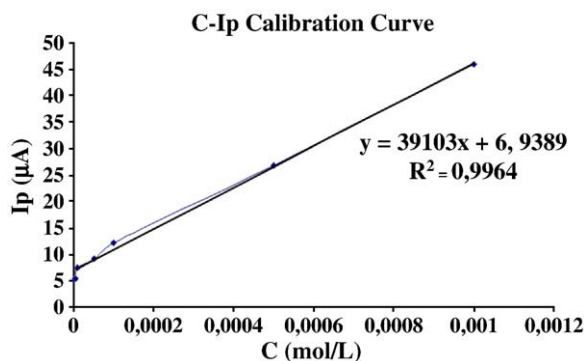


Fig. 13. Standard calibration curve for phenol.

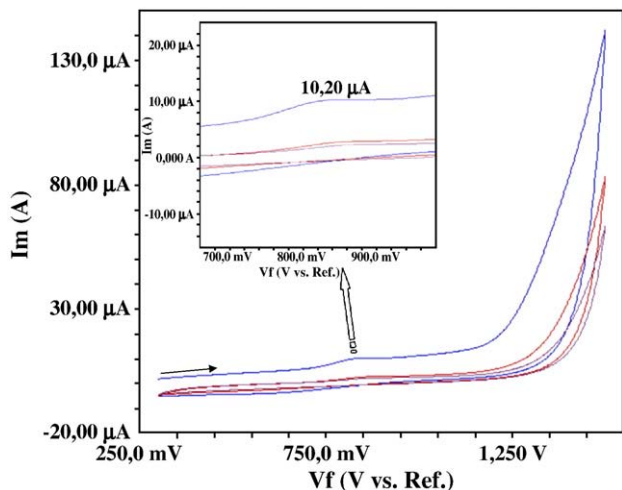


Fig. 14. Phenol binding voltammogram to the PR modified CPE surface for the real samples.

#### 4. Conclusion

The quantification of phenol nearly 20 years is an undiscussable fact. However, the electrochemical quantification of this compound using voltammetric technique is very limited. We tried to show that this recent powerful technique can be applicable for the determination of phenol at low concentrations. Voltammetric techniques are advantageous to the others because they are inexpensive and reliable. Besides all colored and turbid solutions can be easily analyzed using voltammetric techniques. For this reason, we tried to develop a specific sensor electrode for the determination of phenol by modifying CPE surface using procaine in non-aqueous solution. This study was successfully applied to the real samples for phenol determination. The suggested method can also be applied to drinking water, waste water, soil and food samples in which preconcentration might be needed in some cases although we applied to the decayed leaves. This method can be used in the determination of not only for phenol but also for phenol derivatives. Lastly, we developed an electrochemical sensor for this quantification. By using this developed sensor electrode one can easily quantitatively determine phenol at very low concentrations.

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