

## An electrochemical sensor based on multi-walled carbon nanotubes and 4-(((4-mercaptophenyl)imino)methyl) benzene-1,2-diol for simultaneous determination of epinephrine in the presence of acetaminophen

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### Abstract

A carbon paste electrode modified with 4-(((4-mercaptophenyl)imino)methyl)benzene-1,2-diol (MIB) and multi-walled carbon nanotubes (MIB /CNT/CPE) was prepared for determination of epinephrine (EP) in the presence of acetaminophen (AC). Cyclic voltammetry, chronoamperometry, and differential pulse voltammetry (DPV) techniques were used to investigate the modified electrode for the electrocatalytic oxidation of EP and AC in aqueous solutions. The separation of the oxidation peak potential for EP- AC was 200 mV. Under the optimum conditions, the calibration curve for EP was obtained in the range of 1.0 to 25.0  $\mu\text{M}$  and 25.0 to 500.0  $\mu\text{M}$ . The diffusion coefficient for the oxidation of EP at the surface of the modified electrode was calculated as  $5.76 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .

**Keywords:** Acetaminophen, Carbon nanotubes, Carbon paste electrode, Epinephrine.

### 1. Introduction

Carbon paste electrode (CPE) is a specific kind of heterogeneous carbon electrode. This electrode consists of a mixture prepared from carbon powder (such as graphite, glassy carbon and others carbonaceous materials) and a suitable water-immiscible or non-conducting binder (1-3). Carbon paste as an electrode was initially reported in 1958 by Adams (4). Then, a wide variety of modifiers including polymers, enzymes, and nanomaterials (5-8) were used with carbon paste as versatile electrodes. CPEs are widely applicable in electrochemical and electro-analysis studies thanks to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), low cost, convenient preparation, simple surface renewal process, large potential window, easiness of miniaturization (9-11), and the feasibility of incorporating different substances during paste preparation (12, 13). Since carbon

nanotubes (CNTs) were discovered in 1991 (14), numerous studies have been focused on investigating their applications (15-17). Because of the special structure of CNTs, they have several unique properties such as good electrical conductivity, high chemical stability, and extremely high mechanical strength (18, 19). In addition, CNTs electronic behavior reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials (20, 21). These unique properties make CNTs suitable candidates for the modification of electrodes (22-24).

Epinephrine (EP) (also known as adrenaline, adrenalin, or 4,5- $\beta$ -trihydroxy-*N*-methylphenethylamine) is a hormone and a neurotransmitter. Epinephrine is one of the most powerful known vasopressor (causing a rise in blood pressure) drugs. It increases the strength of heart muscle contractions as well as the heart rate and constricts blood vessels and veins. Since it is powerful heart stimulation activity, it is used in emergency to restore heart rhythm in cases of

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shock and in certain cases of cardiac arrest (heart attack), also for treating a number of conditions such as anaphylaxis, and superficial bleeding (25, 26).

There are some methods applied for the determination of EP, such as capillary electrophoresis, high performance liquid chromatography (HPLC), chemiluminescence, flow injection, fluorimetry, and spectrophotometry. EP as an electroactive compound, can also be studied via electrochemical techniques. Some papers showed the electrochemical response of EP on different kinds of electrodes (27-31).

Acetaminophen (AC) contains a phenolic hydroxyl group, which is electrochemically active and can be oxidized. Thus, various modified electrodes have been used to prepare electrochemical sensors for the detection of AC (32-40).

The usefulness of this study lies in the point that AC usage could have a negative impact on heart disease. On the other hand, EP is a powerful heart stimulant. Therefore, the simultaneous measurement of these two compounds is important. In this study, we describe the preparation of a new electrode composed of carbon nanotube paste electrode (CNTPE) modified with 4-(((4-mercaptophenyl)imino)methyl)benzene-1,2-diol (MIB) and investigate its performance for the electrocatalytic determination of EP in aqueous solutions. Moreover, the analytical performance of the modified electrode for quantification of EP in the presence of AC is evaluated.

## 2. Materials and methods

### 2.1. Apparatus and reagents

Morphological characterization was carried out using a transmission electron microscope (TEM, Philips EM 208S at operating voltage of 100 kV) and scanning electron microscope (SEM, VEGA 3 SEM). Electrochemical measurements were performed with a potentiostat/galvanostat Eviumstat. A Metrohm 691 pH/Ion meter was used to read the pH of the buffered solutions. A three-electrode cell was used at  $25 \pm 1$  °C. (CPE), multi-walled carbon nanotube paste electrode (MWCNTPE), and MIB/MWCNTPE were used as working electrodes. An Ag/AgCl (KCl, sat.) electrode and a platinum wire were used as the

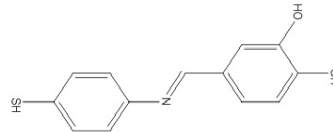
reference and auxiliary electrodes, respectively. All solutions were freshly prepared with double distilled water. IP, PB obtained from Fluka. Multi-walled carbon nanotubes (purity more than 95 %) with o.d. between 5 and 20 nm, i.d. between 2 and 6 nm, and tube length from 1 to 10  $\mu\text{m}$  were prepared from Plasma Chem. Other reagents were of all analytical grade (Merck). Phosphate buffered solutions (PBS), as a 0.1 M solution in the pH range of 2.0-11.0, were prepared by mixing stock solutions of  $\text{H}_3\text{PO}_4\text{-NaH}_2\text{PO}_4$  and  $\text{NaH}_2\text{PO}_4\text{-Na}_3\text{PO}_4$ . Graphite powder (Merck) and high viscosity paraffin oil (DC 350, Merck, density =  $0.88 \text{ g cm}^{-3}$ ) were used as binding agents for graphite pastes.

### 2.2. General procedure for the synthesis of 4-(((4-mercaptophenyl)imino)methyl)benzene-1,2-diol (MIB)

A mixture of 3,4-dihydroxybenzaldehyde (1 mmol), 4-aminobenzenethiol (1 mmol) and acetonitrile (3 mL) was taken in a 5 mL round bottomed flask and heated at 70 °C. After completion of the reaction (observed on a thin-layer chromatography; n-hexane: ethyl acetate 7:3) the prepared mixture was cooled to room temperature. Then, the precipitated product was filtered from the mixture by centrifugation and was purified using column chromatography. The melting point of MIB was obtained as 219-220 °C (Scheme 1).

$^1\text{H}$  and  $^{13}\text{C}$  NMR were recorded in  $\text{DMSO-}d_6$  on a BRUKER DRX-400 AVANCE spectrometer. Fourier transform infrared (FT-IR) spectra were recorded on a BRUKER EQUINOX 55 single beam spectrometer at ambient conditions using KBr disks, with a nominal resolution of 4  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  (ppm) = 3.4 (s, SH), 6.74-6.79 (t, 3 H), 7.08 (s, 3 H), 7.33 (s, 1 H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ )  $\delta$  (ppm) = 115.4, 117.0, 117.2, 123.6, 123.7, 130.0, 144.8, 147.2, 150.2, 157.2, 158.9.



**Scheme 1.** Chemical structure 4-(((4-mercaptophenyl)imino)methyl)benzene-1,2-diol.

IR (KBr):  $\nu=685, 755, 818, 877, 963, 974, 1010, 114, 1165, 1210, 1261, 1289, 1337, 1403, 1460, 1505, 1518, 1575, 1592, 1608, 2485, 2561, 2700, 2854, 2924, 2957, 3070, 3296 \text{ cm}^{-1}$ .

### 2.3. Preparation of the electrode

To obtain the best conditions in the preparation of MIB/MWCNTPE, the ratio of MIB and MWCNTs was optimized. Our results showed that the maximum peak current intensity of isoprenaline and phenobarbital could be obtained. The MIB/MWCNTPE were prepared by dissolving 0.02 g MIB in 1 mL chloromethane, then 0.900 g graphite powder, 0.040 g multiwall carbon nanotubes, and 0.040 g Pt-nanoparticles with was added and mixed in a mortar and pestle. Then, 0.6 mL of paraffin oil were added to the above mixture and mixed for 20 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube. A copper wire was inserted through the opposite end to establish an electrical contact. Prior to the experiment, the surface of the prepared electrode was polished with a fine paper. For comparison, other electrodes were prepared in the same way, such as MIB modified CPE electrode (MIB-CPE) without MWCNT, MWCNT paste electrode (MWCNTPE) without MIB, and unmodified CPE in the absence of MIB and MWCNTPE.

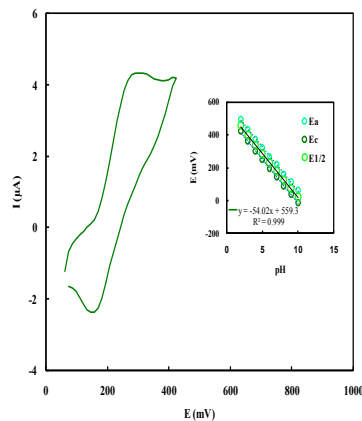
## 3. Results and discussion

### 3.1. Electrochemical properties of modified MIB/MWCNTPE

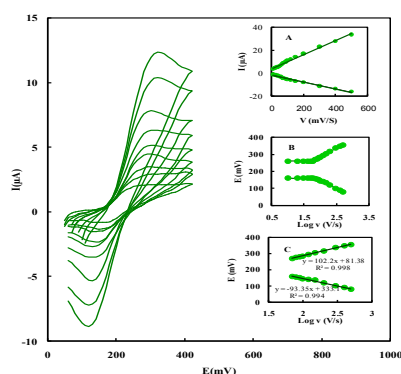
One of the advantages of MIB as an electrode modifier is its insolubility in aqueous media. Therefore, MIB/MWCNTPE was prepared and its electrochemical properties in an aqueous solution (pH 7.0) was investigated using cyclic voltammetry (CV). Figure 1 shows the CV of the modified electrode at scan rate of  $30 \text{ mV s}^{-1}$  in 0.1 M phosphate buffer (pH 7.0). The experimental results showed reproducible, well-defined, anodic and cathodic peaks with  $E_{pa}$ ,  $E_{pc}$ , and  $E^{0'}$  ( $=E_{pa}-\alpha(E_{pa}-E_{pc})$ ) of 0.261, 0.160, and 0.219 V, vs Ag/AgCl/KCl (3.0 M), respectively. The observed peak separation potential,  $\Delta E_p=(E_{pa}-E_{pc})=101 \text{ mV}$ , was greater than the value expected for a reversible system, suggesting that the redox couple

in MIB/CNT/CPE shows quasi-reversible behavior in an aqueous medium. The electrochemistry of MIB molecule is generally pH dependent. Thus, the electrochemical behavior of MIB/CNT/CPE was studied at different pH by CVs. As can be seen in the inset of Figure 1, the formal potential ( $E^{0'}$ ) of MIB/CNT/CPE was pH dependent. Since one straight line was obtained with a slope value of  $-54.0 \text{ mV per pH}$  in the pH ranges of 2.0-10.0, there is a transfer of two electrons and two protons in the redox reaction of MIB in the pH range of 2.0-10.0 (41).

In addition, the effect of the potential scan rate on electrochemical behavior of the MIB/CNT/CPE was studied in 0.1 M phosphate buffer solution. Plots of both anodic and cathodic peak currents ( $I_p$ ) were linearly dependent on  $v$  in the range of 10 to  $500 \text{ mV s}^{-1}$  (Figure 2), indicating that the redox process is not controlled by diffusion. The apparent charge transfer rate constant,  $k_s$ , and the charge transfer coefficient,  $\alpha$ , of a surface-confined redox couple were evaluated through CV experiments and using the variation of anodic and cathodic peak potentials with the logarithm of the scan rate, according to the procedure of Laviron (42). We realized that the  $E_p$  values were proportional to the logarithm of the potential scan rate, for scan rates higher than  $80 \text{ mV s}^{-1}$  (Figure 2C). The slopes of the plots can be used to extract the kinetic parameter  $\alpha$  (anodic transfer coefficient). The slope of the linear segment was equal to  $2.303RT/(1-\alpha_a)n\alpha F$  for the anodic peaks and  $2.303RT/\alpha_c n_a F$  for the cathodic peaks. The calculated value



**Figure 1.** Cyclic voltammogram obtained at MIB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) at a scan rate of  $30 \text{ V s}^{-1}$ . The inset shows a plot of  $E^{0'}$  of MIB/CNT/CPE versus pH.



**Figure 2.** Cyclic voltammograms obtained at MIB/CNT/CPE in 0.1 M phosphate buffer (pH 7.0) at various scan rates from 10 to 500  $\text{mVs}^{-1}$ , insets: (A) variations of  $I_p$  versus scan rate, (B) variation of  $E_p$  versus the logarithm of scan rate, and (C) variation of  $E_p$  versus the logarithm of scan rate for scan rates higher than 80  $\text{mVs}^{-1}$ .

for the average transfer coefficient ( $\alpha$ ) was 0.41. The following equation can be used to determine the electron transfer rate constant between the modifier (MIB) and CPE:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log(RT/\alpha F v) - \alpha(1-\alpha) n_a F \Delta E_p / 2.3RT \quad (\text{Eq. 1})$$

The value of  $k_s = 2.25 \text{ s}^{-1}$  was evaluated using (Eq. 1).

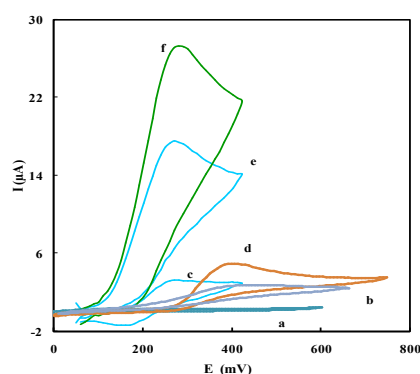
An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp (43). According to this method, the peak current is related to the surface concentration of the electroactive species,  $\Gamma$ , by the following equation:

$$I_p = n^2 F^2 A \Gamma v / 4RT \quad (\text{Eq. 2})$$

where  $n$  represents the number of electrons involved in the reaction,  $A$  is the surface area ( $0.0962 \text{ cm}^2$ ) of the electrode,  $\Gamma$  ( $\text{mol cm}^{-2}$ ) is the surface coverage, and the other symbols have their usual meanings. Based on the slope of the anodic peak currents versus the scan rate (Figure 2A), the calculated surface concentration was  $=1.7 \times 10^{-9} \text{ mol cm}^{-2}$  for  $n=2$ .

### 3.2. Electrocatalytic oxidation of EP at a MIB/CNT/CPE

The utility of the modified electrode for oxidation of EP was evaluated by cyclic voltammetry. Figure 3 depicts the CV responses of MIB/CNT/CPE, MIB/CPE, CNT/CPE, and bare CPE in 0.1 M phosphate buffer (pH 7.0) in the absence and presence of EP. As seen, while the anodic peak

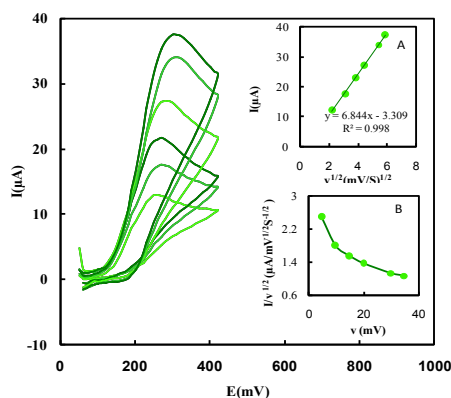


**Figure 3.** CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0), (b) unmodified CPE in 0.20 mM EP, (c) MIB/CNT/CPE in 0.1 M PBS, (d) CNT/CPE in 0.20 mM EP, (e) MIB/CPE in 0.2 mM EP, and (f) MIB/CNT/CPE in 0.2 mM EP. In all cases, the scan rate was  $10 \text{ mVs}^{-1}$ .

potential for EP oxidation at the CNT/CPE (curve d) and unmodified CPE (curve b) were 385 and 460 mV, respectively, the corresponding potential at MIB/CNT/CPE (curve f) and MIB/CPE (curve e) was 272 mV. These results indicated that the peak potential for EP oxidation at the MIB/CNT/CPE and MIB/CPE electrodes was shifted by  $\sim 188 \text{ mV}$  toward negative values compared to CNT/CPE and unmodified CPE, respectively. However, MIB/CNT/CPE showed much higher anodic peak current for the oxidation of EP compared to MIB/CPE, indicating that the combination of CNTs and the mediator (MIB) significantly improved the performance of the electrode toward EP oxidation. In fact, MIB/CNT/CPE in the absence of EP exhibited a well-behaved redox reaction (Figure 3, curve c) in 0.1 M PBS (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 0.20 mM EP (curve f), which can be related to the strong electrocatalytic effect of the MIB/CNT/CPE towards this compound.

The effect of scan rate on the electrocatalytic oxidation of  $200 \mu\text{M}$  EP at the MIB/CNT/CPE was investigated by cyclic voltammetry. The oxidation peak potential was shifted with increasing scan rates towards a more positive potential (Figure 4), confirming the kinetic limitation of the electrochemical reaction. Based on inset A of Figure 4, the anodic oxidation current of EP was proportional to the square root of the scan rate showing that the reaction was controlled by diffusion (23). A plot of the sweep rate normalized current ( $I_p/v^{1/2}$ ) versus sweep rate (inset B of Figure 4) exhibited the characteristic shape typical of an EC'



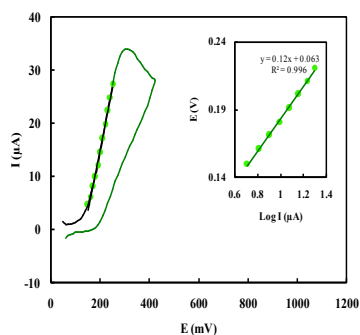


**Figure 4.** Cyclic voltammograms obtained at MIB/CNT/CPE in 0.1 M phosphate buffer (pH 7.0) containing 0.200 mM EP at scan rates 5, 10, 15, 20, 30 and 35  $\text{mVs}^{-1}$ , (A) variation of the electrocatalytic currents vs. the square root of the scan rate (B) variation of the scan rate normalized current ( $I_p/v$ ) with scan rate.

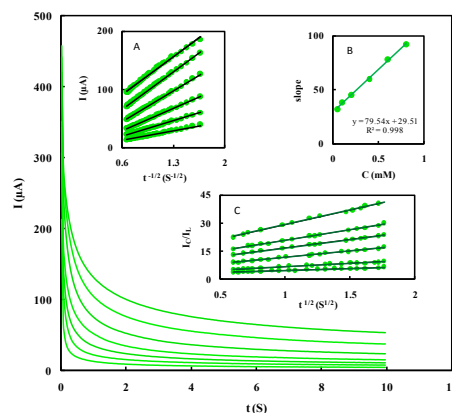
reaction (20). Figure 5 showed the cyclic voltammogram, at 30  $\text{mVs}^{-1}$ , of MIB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) containing 200  $\mu\text{M}$  EP. The inset of Figure 5 showed a Tafel plot that was drawn from points of the Tafel region of the linear sweep voltammogram. The Tafel slope of 120.0  $\text{mV decade}^{-1}$  obtained in this case, calculates a charge transfer coefficient of  $\alpha=0.37$ .

### 3.3. Chronoamperometric measurements

Chronoamperometric measurements of EP at MIB/CNT/CPE were carried out by setting the working electrode potential at 0.35 V vs. Ag/AgCl/KCl (3.0M) for the various concentrations of EP in buffered aqueous solutions (pH 7.0) (Figure 6). For an electroactive material (EP in this case) with a diffusion coefficient of  $D$ , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell



**Figure 5.** Cyclic voltammogram obtained at MIB/CNT/CPE in 0.1 M phosphate buffer (pH 7.0) containing 200  $\mu\text{M}$  EP at a scan rate of 30  $\text{mV s}^{-1}$ , the points are the data used in the Tafel plot. The inset indicates the Tafel plot derived from the cyclic voltammogram.



**Figure 6.** Chronoamperograms obtained at MIB/CNT/CPE in 0.1M phosphate buffer solution (pH 7.0) for concentrations of EP: 0.0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 mM. Insets: A) plots of  $I$  vs.  $t^{1/2}$  obtained from chronoamperograms, B) plot of the slope of the straight lines against the EP concentration with the linear equation of slope ( $\mu\text{A s}^{-1/2}$ )= $79.54 C$  (mM)+29.51 and C) dependence of  $I_C/I_L$  on  $t^{1/2}$  derived from the data of chronoamperograms.

equation (41).

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (\text{Eq. 3})$$

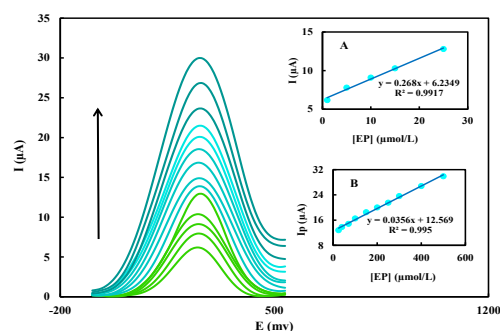
Where  $D$  is the diffusion coefficient ( $\text{cm}^2\text{s}^{-1}$ ) and  $C_b$  is the bulk concentration ( $\text{mol cm}^{-3}$ ) of EP. The experimental plots of  $I$  versus  $t^{1/2}$  for different concentrations of EP are depicted in Figure 6 A. The slopes of the resulting straight lines were then plotted versus the EP concentration (inset B of Figure 6). Based on the resulting slope of inset B of Figure 6 and Cottrell equation, the mean value of the  $D$  was found to be ( $5.76 \times 10^{-5} \text{cm}^2\text{s}^{-1}$ ). Chronoamperometry can also be used to assess the catalytic rate constant,  $k$ , for the reaction between EP and the MIB/CNT/CPE, according to the method of Galus (44).

$$I_{\text{Cat}}/I_L = \gamma^{1/2} [\pi^{1/2}/2 \text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \quad (\text{Eq. 4})$$

Where  $I_C$  is the catalytic current of EP at the MIB/CNT/CPE,  $I_L$  is the limited current in the absence of EP and  $\gamma = kC_b t$  ( $C_b$  is the bulk concentration of EP) is the argument of the error function. In the cases where  $\gamma$  exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_{\text{Cat}}/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(KC_b t)^{1/2} \quad (\text{Eq. 5})$$

Where  $t$  is the time elapsed. Based on the slope of the  $I_C/I_L$  vs.  $t^{1/2}$  plot,  $k$  can be obtained for a given EP concentration. Such plots obtained from the chronoamperograms in Figure 6 are shown in inset C of this figure. Based on the values of the slopes, the average value of  $k$  was found to



**Figure 7.** DPVs of DBC-IL/CNPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of EP. The numbers of 1–14 correspond to: 1, 5, 10, 15, 20, 40, 70, 100, 150, 200, 300, 400, and 500  $\mu\text{M}$  of EP. Insets show the plot of the electrocatalytic peak current as a function of EP concentration in the ranges of (A) 1.0 - 25.0  $\mu\text{M}$ , (B) 25.0–500.0  $\mu\text{M}$ .

be  $k=5.973 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ . The value of  $k$  explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of EP at the surface of MIB/CNT/CPE.

### 3.4. Calibration plot and detection limit

Differential pulse voltammetry (DPV) method was used to determine the concentration of EP because DPV is a powerful electrochemical technique that can be applied in analytical measurements (Figure 7). The responses were linear with EP concentrations in two linear segments (equations of  $I (\mu\text{A})=0.267 C_{\text{EP}}(\mu\text{M})+6.230$  for first segment in the ranges of EP concentration from 1.0 to 25.0  $\mu\text{M}$  and  $I (\mu\text{A})=0.035 C_{\text{EP}}(\mu\text{M})+12.56$  for second segment in the ranges of EP concentration from 25.0 to 500.0  $\mu\text{M}$ ). From the analysis of data, we estimated that the lower limit of detection of EP is approximately 0.25  $\mu\text{M}$ . This value is comparable with values reported by other research groups for electrocatalytic oxidation of EP at the surface of chemically modified electrodes by other mediators (Table 1).

### 3.5. Simultaneous determination of EP and AC

**Table 1.** Comparing various reports of some modified electrodes used in the electrocatalysis of EP with the present work.

Electrode	aModifier	Method	pH	Peak potential Shift (mV)	Limit of detection (M)	Dynamic Range (M)	Reference
CPE	EBNBH	Voltammetry	7.0	160	$2.16 \times 10^{-7}$	$7.0 \times 10^{-7}$ - $1.2 \times 10^{-3}$	(27)
CPE	BBNBH	Voltammetry	8.0	270	$2.0 \times 10^{-7}$	$1.0 \times 10^{-6}$ - $6.0 \times 10^{-4}$	(31)
CPE	Mo (VI) complex	Voltammetry	7.0	125	$4.9 \times 10^{-8}$	$9.0 \times 10^{-8}$ - $7.5 \times 10^{-4}$	(45)
CPE	5ADMB	Voltammetry	7.0	160	$8.8 \times 10^{-8}$	$4.0 \times 10^{-7}$ - $9.0 \times 10^{-4}$	(46)
CPE	DOH	Voltammetry	7.0	280	$8.0 \times 10^{-7}$	$1.0 \times 10^{-5}$ - $6.0 \times 10^{-3}$	(47)
CPE	Iron phthalocyanine	Voltammetry	4.0	100	$5.5 \times 10^{-7}$	$1.0 \times 10^{-6}$ - $13.0 \times 10^{-4}$	(48)
GCE	OMC	Voltammetry	7.0	170	$2.0 \times 10^{-7}$	$5 \times 10^{-7}$ - $2 \times 10^{-4}$	(49)
GCE	poly CM	Voltammetry	8.0	180	$5.0 \times 10^{-8}$	$4.97 \times 10^{-6}$ - $2.31 \times 10^{-4}$	(50)
GCE	EPAP	Voltammetry	7.4	280	$6.5 \times 10^{-9}$	$1.0 \times 10^{-8}$ - $8.0 \times 10^{-5}$	(51)
Gold Electrode	4-NBA	Voltammetry	5.5	270	$37 \times 10^{-9}$	$1.0 \times 10^{-8}$ - $2.0 \times 10^{-6}$	(52)
CPE	MIB	Voltammetry	7.0	188	$2.5 \times 10^{-8}$	$1.0 \times 10^{-6}$ - $5.0 \times 10^{-4}$	This Work

a Where EBNBH: 2,2'-[1,2-ethanediylbis-(nitriloethylidyne)]-bis-hydroquinone, BBNBH: 2,2'-[1,2 butanediylbis-(nitriloethylidyne)]-bis-hydroquinone. Mo (VI) complex: Molybdenum (VI) complex, 5ADMB: 5-amino-3',4'-dimethoxy-biphenyl-2-ol, DOH: 2,2'-[3,6-dioxo-1,8-octanediybis(nitrilo-ethylidyne)]-bis-hydroquinone, OMC: Ordered mesoporous carbon, poly CM: poly curcumin (1,7 Bis ((4- hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 dione), EPAP: electropolymerization of p-aminophenol, 4-NBA: 4-nitrothiophenol/4-mercaptobenzoic acid, MIB: 4-(((4-mercaptophenyl)imino)methyl)benzene-1,2-dio.

The main objective of this study was to detect EP and AC simultaneously using MIB/CNT/CPE. This was performed by simultaneously changing the concentrations of EP and AC, and recording the SWVs. The DP voltammetric results show two-well-defined anodic peaks at potentials of 271 and 470 mV corresponding to the oxidation of EP and AC, respectively, indicating that simultaneous determination of these compounds is feasible at the MIB/CNT/CPE, as shown in Figure 8. The sensitivity of the modified electrode towards the oxidation of EP was found to be  $0.041 \mu\text{A}$ . This is very close to the value obtained in the absence of AC ( $0.035 \mu\text{M}^{-1}$ , see Section 3.4), indicating that the oxidation processes of these compounds at the MIB/CNT/CPE were independent and therefore, simultaneous determination of their mixtures was possible without significant interferences.

### 3.6. Determination of EP in the real sample

In order to assess the applicability of the proposed electrode, the concentration of EP in an injection sample with a specified content of  $1.0 \text{ mg mL}^{-1}$  was determined using the calibration curve for this compound. The EP injection solution was diluted with water. Then different amounts of diluted solution were transferred in to a series of 10 mL volumetric flasks and diluted to the mark with PBS (pH=7.0). A 10 mL aliquot of this test solution was placed in the electrochemical cell. The potential was controlled between 0.0 and 0.5 V and  $I_{p_a}$  was measured at the oxidation peak of EP. The average concentration of EP in the injection was found to be  $0.99 \text{ mg mL}^{-1}$ , which is in good agreement with the specified value. Afterwards, different standard concentrations of EP were added to the diluted EP injection solution and the measurements were done. The results are given in Table 2.

### 3.7. Interference studies

The influence of various species interfering with the determination of EP was studied under optimum conditions. The tolerance limit was taken as the maximum concentration of the interfering that caused an error of less than  $\pm 5\%$  in the determination of EP. According to the results,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , l-lysine, glucose, tryptophan, N-acetyl cysteine, and uric acid did not show interference

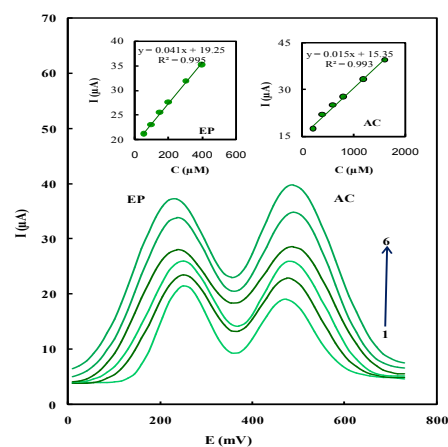
in the determination of EP.

### 3.8. Repeatability, reproducibility, and stability of MIB/CNT/CPE

DPV was employed for checking a repeatable response for five measurements with two concentrations of EP. The RSD was obtained as 1.3 and 1.9 % for 200 and 400  $\mu\text{M}$  of EP, respectively. DPV experiments of five separately prepared MIB/CNT/CPE was examined for testing the reproducibility. The calculated RSD for DPV peak current is about 2.1% for different concentrations of EP, indicating that reproducibility of the preparation is excellent. The long-term stability of the MIB/CNT/CPE was tested over a three-week period. In the DPVs measurements, the peak potential for EP oxidation was unchanged and the current signals showed less than 2.2% decrease relative to the initial response after three weeks.

## 4. Conclusion

The results obtained in this work demonstrated the potentiality of the MIB/CNT/CPE for simultaneous determination of EP and AC. The modified electrode exhibited highly electrocatalytic activity for the oxidation of EP at pH 7.0, whereas the peak potential of EP was shifted by



**Figure 8.** Differential pulse voltammograms obtained MIB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of EP and AC (from inner to outer) mixed solutions of 50.0+200.0, 100.0+400.0, 150.0+600.0, 200.0+800.0, and 300.0+1200.0  $\mu\text{M}$  respectively. Insets: A) plot of the peak currents as a function of EP concentration with the regression equation of  $I (\mu\text{A}) = 0.041 \text{ CEP}(\mu\text{M}) + 19.25$  and B) Plot of the peak currents as a function of AC concentrations with the regression equation of  $I (\mu\text{A}) = 0.015 \text{ CAC} (\mu\text{M}) + 15.85$ . Scan rate: 20 mV s<sup>-1</sup>, modulation time: 0.05 s, pulse amplitude: 0.02 V.

**Table 2.** Determination of EP in real sample (n=3).

No.	EP injection ( $\mu\text{M}$ )	Found ( $\mu\text{M}$ )	Recovery (%)	RSD (%)
1	5.0	4.9	98.0	2.5
2	10.0	10.2	102.0	1.9
3	15.0	14.8	98.9	1.4
4	20.0	20.1	100.5	2.7

188 mV to a less positive potential at the surface of the MIB/CNT/CPE. The modified electrode displayed higher selectivity in voltammetric measurements of EP and AC in their mixture solution.

The high sensitivity and selectivity, sub-micro-molar detection limit, high reproducibility, and ease of preparation and regeneration of the electrode surface by simple polishing make the designed electrode very suitable for the determination of EP in pharmaceutical and clinical prepara-

tions.

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### Conflict of Interest

None declared.

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