

Multi-wall carbon nanotubes and TiO₂ as a sensor for electrocatalytic determination of epinephrine in the presence of *p*-chloranil as a mediator

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Abstract In this work, we describe an electrochemical method using *p*-chloranil as a mediator and multi-wall carbon nanotube and TiO₂ as sensors for sensitive determination of epinephrine (EP) in aqueous solution at pH=10.0. It has been found that under optimum condition (pH 10.0) in cyclic voltammetry, the oxidation of EP occurred at a potential about 171 mV less positive than that unmodified carbon nanotube paste electrode. The diffusion coefficient (*D*) and the kinetic parameters, such as electron transfer coefficient, (α) and heterogeneous rate constant (k_h) for EP were also determined using electrochemical approaches. The electrocatalytic currents increase linearly with the EP concentration over the range 0.6–135 μ M. The detection limits for EP will be equal to 0.25 μ M. The relative standard deviation percentage values for 10.0 and 15.0 μ M EP were 1.7% and 1.9%, respectively. Finally, this modified electrode was also examined as a selective, simple, and precise new electrochemical sensor for the determination of EP in real sample such as urine and epinephrine injection solution.

Keywords Epinephrine determination · Carbon nanotubes and TiO₂ paste electrode · *p*-Chloranil · Electrocatalytic · Voltammetry

Introduction

Epinephrine or adrenaline is a hormone and neurotransmitter [1]. When produced in the body, it increases

heart rate, contracts blood vessels and dilates air passages, and participates in the “fight or flight” response of the sympathetic nervous system [2]. It is a catecholamine, a sympathomimetic monoamine produced only by the adrenal glands from the amino acids phenylalanine and tyrosine. Adrenaline was first synthesized by Friedrich Stolz and Henry Drysdale Dakin, independently, in 1904 [3]. Adrenaline is a powerful action, “fight or flight,” hormone and also plays a central role in the short-term stress reaction. Adrenaline’s actions are mediated through adrenergic receptors. Adrenaline is a non-selective agonist of all adrenergic receptors. It activates α_1 , α_2 , β_1 , and β_2 receptors to different extents [4]. However, adrenaline is used as a drug to treat cardiac arrest.

Several methods have been described in the literature for the determination of EP such as HPLC [5–8], spectrophotometry [9, 10], capillary electrophoresis [11], chemiluminescence [12], fluorimetry [13–15], and some electrochemical methods [16–20].

Since their initial discovery by Iijima in 1991, carbon nanotubes (CNTs), as one of the most interesting carbon materials, have attracted considerable attention of researchers over the past years [21]. In the electroanalytical field, as they can promote electron transfer reactions, they are widely used as electrode materials and to prepare sensors [22–24].

Therefore, in continuation of our studies concerning the preparation of chemically modified electrodes [25–28], we describe the use of multi-wall carbon nanotube and TiO₂ as sensors and *p*-chloranil as a mediator for the electro-oxidation of EP in aqueous media. Also, the suitability of the modified multi-wall carbon nanotube paste electrode is discussed by cyclic voltammetry, double potential step chronoamperometry. Finally, ability of this method study to

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determination of EP in real samples such as urine and epinephrine injection solution.

Experimental part

Apparatus and reagents

All electrochemical measurements were performed using BHP 2063+ Electrochemical Analysis System, Behpajooh, Iran potentiostat/galvanostat coupled with a Pentium IV personal computer connected to a HP laser jet 6L printer. An Ag/AgCl/KCl 3 M, a platinum wire, and a *p*-chloranil-modified multi-wall carbon nanotubes and TiO₂ paste electrode (*p*-CMMWCNTPE) were used as reference, auxiliary and working electrodes, respectively. A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. Graphite fine powder, paraffin oil, and reagents were analytical grade from Merck. EP was purchased from Fluka. Multi-walled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm and tube length from 0.5 to 200 nm were prepared from Nanostructured & Amorphous Materials (USA). Universal buffer solutions (boric acid, phosphoric acid, acetic acid, and sodium hydroxide, 0.04 M) with different pH levels were used to study the influence of pH.

Preparation of the electrode

Modified carbon nanotubes and TiO₂ paste electrodes were prepared by dissolve 0.01 g of *p*-chloranil in diethyl ether and hand mixing with 84 times its weight of graphite powder, 0.05 g TiO₂, and ten times its weight of carbon nanotubes with a mortar and pestle. The solvent was evaporated by stirring. A 70:30 (*w/w*) mixture of *p*-chloranil spiked carbon nanotubes powder and paraffin oil was blended by hand mixing for 20 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube. Electrical contact was made by inserting a copper wire into the glass tube at the back of the mixture. When necessary, a new surface was obtained by pushing an excess of paste out of the tube and polishing it on a weighing paper. Unmodified carbon paste was prepared in the same way without adding *p*-chloranil, TiO₂, and carbon nanotubes to the mixture and was used for comparison purposes.

Recommended procedure

The modified multi-wall carbon nanotubes paste electrode was polished with a white and clean paper. To prepare a blank solution, 10.0 mL of universal buffer solution

(pH 10.0), was transferred into an electrochemical cell. The initial and final potentials were adjusted to -0.10 and $+0.25$ V vs. Ag/AgCl, respectively. The differential pulse voltammetry (DPV) was recorded to give the blank signal and labeled as I_{pb} . Then different amounts of EP solution were added to the cell, using a micropipette, and the DPV was recorded again to get the analytical signal (I_{ps}). Calibration curves were constructed by plotting the catalytic peak current vs. the EP concentration.

Results and discussion

Electrochemistry of *p*-chloranil

The electrochemical properties of modified electrode were studied by cyclic voltammetry in buffer solution (pH 10.0). Figure 1 (inset) shows the cyclic voltammograms of this electrode at various scan rates, ($\nu=10\text{--}150$ mV s⁻¹). The experimental results show well-defined and reproducible anodic and cathodic peaks related to *p*-CA_(Red)/*p*-CA_(Ox) redox couple with quasi-reversible behavior, with peak separation potential of ΔE_p ($E_{pa}-E_{pc}=130$ mV). These cyclic voltammograms were used to examine the variation of the peak currents vs. scan rates of potential. The plot of the peak current was linearly dependent on $\nu^{1/2}$ with a correlation coefficient of 0.9964 at the all scan rates (Fig. 1). This behavior indicates that the nature of redox process is diffusion controlled [29].

pH optimization

The electrochemical behavior of EP and *p*-chloranil are dependent on the pH value in the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of

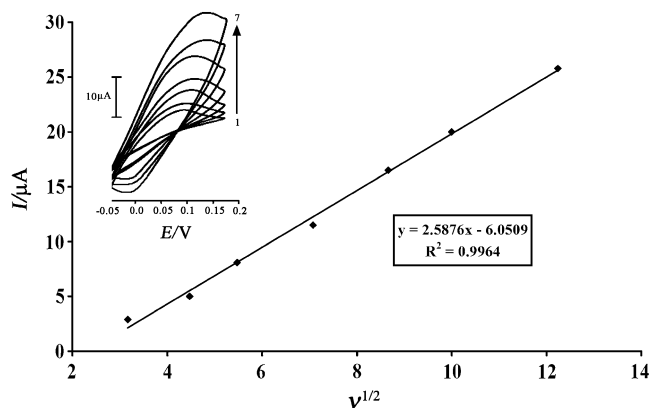


Fig. 1 Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of *p*-CMMWCNTPE. Inset shows cyclic voltammograms of at various scan rates: 1 10, 2 20, 3 30, 4 50, 5 75, 6 100, and 7 150 mV s⁻¹ in 0.04 M buffer solution (pH 10.0)

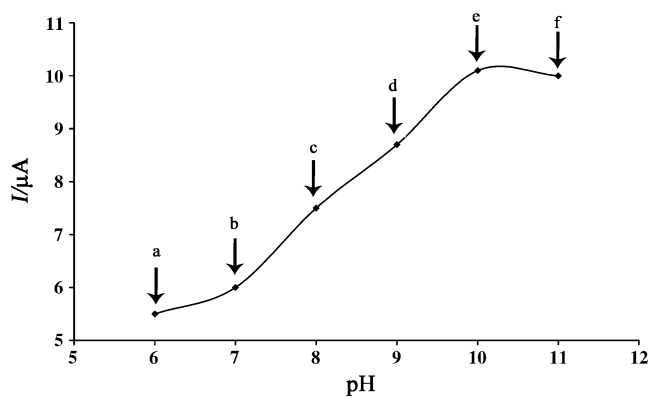


Fig. 2 Current–pH curve for electrooxidation of 300 μM EP in 0.04 M universal buffer solution with various pH values: *a* 6.00, *b* 7.00, *c* 8.00, *d* 9.00, *e* 10.00, and *f* 11.00 at the surface of *p*-CAMMWCNTPE as scan rate 10 mV s^{-1}

EP. Thus, we studied the electrochemical behavior of EP in 0.04 M universal buffer in different pH values ($6.00 < \text{pH} < 11.00$) at the surface of *p*-CAMMWCNTPE by cyclic voltammetry. The results showed that the maximum value of the oxidation peak current was gradually reached with increasing in pH up to 10.0 (see Fig. 2). The peak potential was also found to be positively shifted with increasing pH. The results indicated that proton could participate in the electrochemical reaction. As observed, in the acidic media, the drugs do not considerably affect the peak current of *p*-chloranil because the oxidation of *p*-chloranil is difficult to occur under this condition. Furthermore, as stated before, $\text{p}K_{\text{a}2}$ of the reduced form of CA is about 8.00. The suitable form of the reduced form of *p*-chloranil, which undergoes oxidation on the paste electrode, is the anionic form. Thus, oxidation of the reduced form to the oxidized form of *p*-chloranil is easier in basic media. Therefore, we observed a better electrocatalytic behavior for *p*-chloranil in the basic solution. When the pH level was increased to above 10.0, no enhancement was observed in the peak current due to changes in drugs' structures. Therefore, pH 10.00 was chosen as the optimum pH for electrocatalysis of EP at the surface of *p*-CAMMWCNTPE.

Stability and reproducibility

The repeatability and stability of the *p*-CAMMWCNTPE was investigated by cyclic voltammetry measurements of 80 μM EP in optimum condition (pH 10.0; scan rate 10 mV s^{-1}). The relative standard deviation (RSD) for ten successive assays is 1.9%. When using five different electrodes in presence of 80 μM EP in 0.04 M buffer solution (pH 10.0), the RSD for five measurements is 2.1% by cyclic voltammetry. When stored in a laboratory, the modified electrode retains 95% of its initial response after a

week and 91% after 30 days. These results indicate that *p*-CAMMWCNTPE has good stability and reproducibility and could be used for EP measurements.

Catalytic effect

Figure 3 depicts the cyclic voltammetric responses for the electrochemical oxidation of 300 μM EP at *p*-CAMMWCNTPE (curve *c*), *p*-chloranil-modified carbon paste electrode (*p*-CAMCPE; curve *b*), carbon nanotubes paste electrode (CNPE; curve *d*), and a bare CPE (curve *e*). As can be seen, the anodic peak potential for the oxidation of EP at *p*-CAMMWCNTPE (curve *c*) and *p*-CAMCPE (curve *b*) is about 60 mV, while it is about 231 mV at the CNPE the bare CPE. From these results, it is concluded that the best electrocatalytic effect for EP oxidation is observed at *p*-CAMMWCNTPE. The results show that the peak potential of oxidation at *p*-CAMMWCNTPE shifted by about 171 mV toward the negative values compared with those at a CNPE and bare CPE. Similarly, when we compared EP oxidation behaviors at the *p*-CAMMWCNTPE and *p*-CAMCPE, we found a dramatic enhancement of the anodic peak current at *p*-CAMMWCNTPE relative to the value obtained at the *p*-CAMCPE. In other words, the data obtained clearly show that the combination of carbon nanotubes, TiO_2 , and the mediator (*p*-chloranil) definitely improves the characteristics of EP oxidation.

The effect of scan rate on the electrocatalytic oxidation of 350 μM EP at the *p*-CAMMWCNTPE was investigated by cyclic voltammetry (Fig. 4, insert). The oxidation peak potential shifted with increasing scan rate towards more positive potentials, confirming the kinetic limitation of the electrochemical reaction. In addition, a plot of peak height

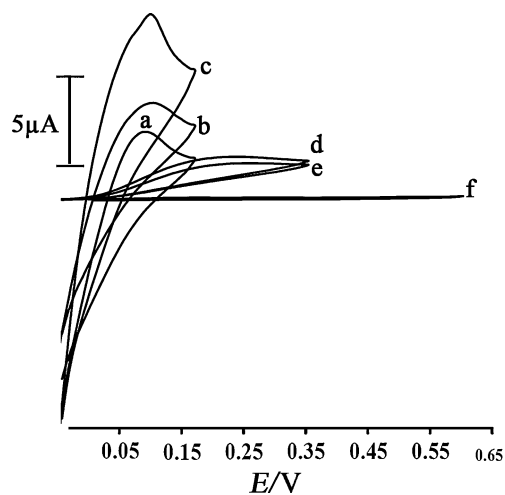


Fig. 3 Cyclic voltammograms of 0.04 M universal buffer (pH 10.0) at scan rate of 10 mV s^{-1} for *a* and *c* in the absence and the presence of 300 μM EP at *p*-CAMMWCNTPE, respectively; *b* as *c* at *p*-CAMCPE; *d* as *c* at CNPE; *e* as *b* at CPE and *f* as *a* without the mediator

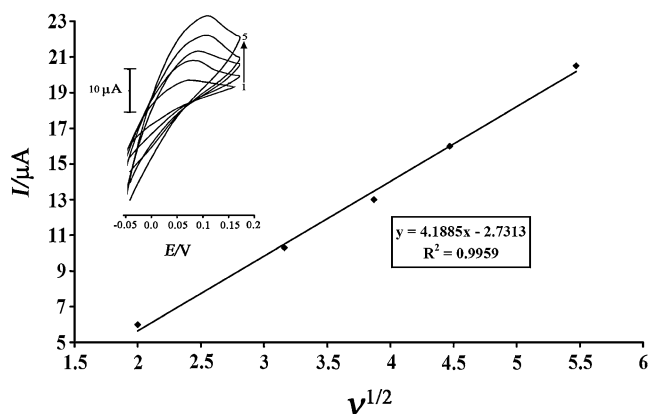


Fig. 4 Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of EP at the surface of *p*-CAMMWNTPE. Inset shows cyclic voltammograms of 350 μM EP at various scan rates: 1 4, 2 10, 3 15, 4 20, and 5 30 mV s^{-1} in 0.04 M universal buffer solution (pH 10.0)

(I_p) against the square root of the scan rate ($\nu^{1/2}$) was linear in range of 4–30 mV s^{-1} (Fig. 4), suggesting that at a sufficient over potential, the process is diffusion rather than surface controlled.

Figure 5 shows a Tafel plot that was drawn from data of the rising part of the current–voltage curve recorded at a scan rate of 30 mV s^{-1} . This part of the voltammogram, known as a Tafel region, is affected by electron transfer kinetics between the substrate (EP) and the surface confined *p*-chloranil, assuming the deprotonation of substrate as a sufficiently fast step. In this condition, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot. Using this data, we obtained α equal to 0.68.

Chronoamperometric measurements

Figure 6 shows chronoamperometric measurements of EP at *p*-CAMMWNTPE. Figure 6 represents the current–time

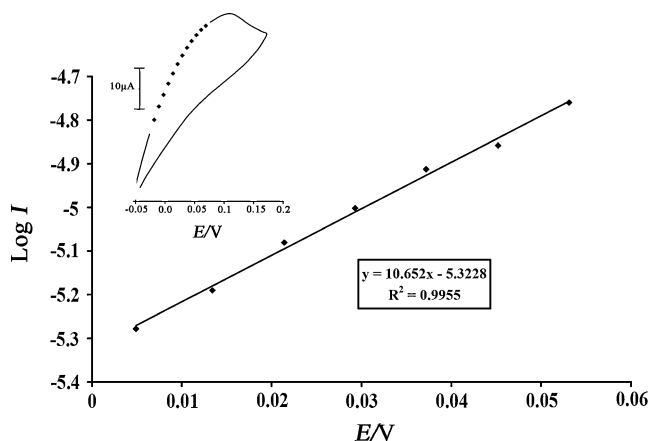


Fig. 5 Tafel plot *p*-CAMMWNTPE in 0.04 M universal buffer (pH 10.0) at a scan rate of 30 mV s^{-1} in the presence of 350 μM EP

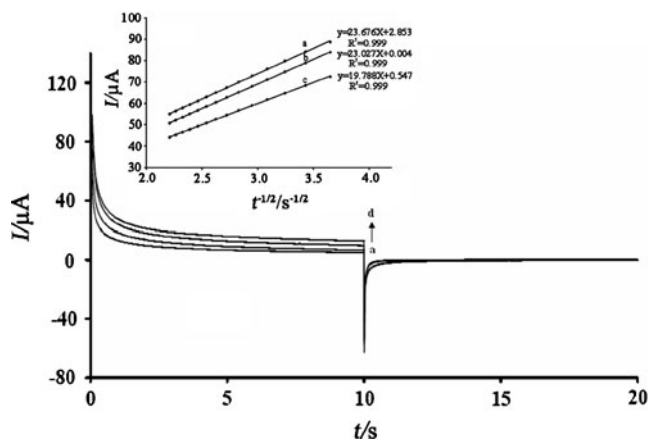


Fig. 6 Chronoamperograms of 0.04 M universal buffer (pH 10.0) at *p*-CAMMWNTPE for **a** in the absence of EP and for **b** 100 μM ; **c** 300 μM and **d** 700 μM of EP. Inset shows Cottrell's plot for the data from the chronoamperograms

profiles obtained by setting the working electrode potential at -0.05 and 0.17 mV for various concentrations of EP. For an electroactive material (EP in this case) with a diffusion coefficient of D , the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation [30]:

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

where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Under the diffusion control condition, a plot of I vs. $t^{-1/2}$ will be linear, and from the slope, the value of D can be calculated. Inset in Fig. 6 shows the experimental plots with the best fits for different concentration of EP employed. The mean value of the D was found to be $1.602 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$ (Fig. 6).

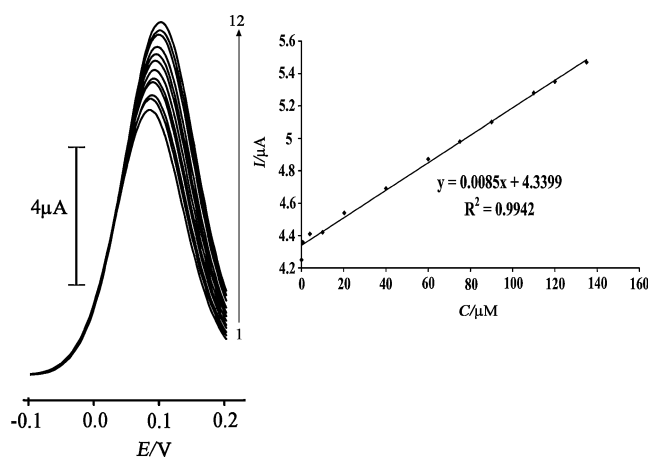


Fig. 7 Differential pulse voltammograms of *p*-CAMMWNTPE in the buffer solution (pH 10.0) containing different concentration of EP. The I_{p-12} corresponds to 0, 0.6, 4.0, 10.0, 20.3, 40, 60, 75, 90, 110, 120, and 135 μM EP. Inset shows the plots of electrocatalytic peak current as a function of EP concentration

Table 1 Interference study for the determination of 50.0 μM EP under the optimized conditions

| Species | Tolerant limits ($W_{\text{Substance}}/W_{\text{Captopril}}$) |
|---|---|
| Glucose, Sacarose, Lactose, Fructose, K^+ , Na^+ , CO_3^{2-} , Ca^{2+} , Mg^{2+} , ClO_4^- , SO_4^{2-} , F^- , Cl^- | 600 |
| Urea | 300 |
| <i>L</i> -Threonine, <i>L</i> -Phenylalanine, Histidine Glycine, Methionine, Alanine, Tryptophane | 200 |
| Starch | Saturation |
| Ascorbic Acid | 2 |

Although ascorbic acid is interference, but interference from ascorbic acid can be minimized by using ascorbic oxidase enzyme

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k_h for the reaction between EP and the *p*-CAMMWCNTPE according to the method of Galus [31]:

$$I_C/I_L = \gamma^{1/2}[\pi^{1/2}\text{erf}(\gamma^{1/2}) + \exp(-\gamma)/\gamma^{1/2}] \tag{2}$$

where I_C is the catalytic current of EP at the *p*-CAMMWCNTPE, I_L is the limiting 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 γ exceeds two the error function is almost equal to 1 and therefore the above equation can be reduced to:

$$I_C/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(k_h C_b t)^{1/2} \tag{3}$$

where t is the time elapsed in seconds. The above equation can be used to calculate the rate constant of the catalytic process k_h . Based on the slope of the I_C/I_L vs. $t^{1/2}$ plot, k_h can be obtained for a given EP concentration. From the values of the slopes, an average value of k was found to be $k_h = 6.77 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$. The value of k_h explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of EP at the surface of *p*-CAMMWCNTPE.

Determination of epinephrine

Since DPV has a much higher current sensitivity than cyclic voltammetry, it was used for determination of EP. Results show a linear segment for EP: for 0.6–135 μM of EP, the regression equation was $I_p(\mu A) = (0.0085 \pm 0.0001)C_{EP} + (4.3399 \pm 0.02)$ ($R^2 = 0.9942$), $n = 12$ where C_{EP} is μM concentration of EP (Fig. 7).

Table 2 Determination of EP in urine sample

| Sample | Added (μM) | Expected (μM) | Founded (μM) ^a | RSD (%) |
|--------|------------|---------------|---------------------------|------------------|
| 1 | Urine | – | – | <detection limit |
| 2 | 10.0 | 10.0 | 10.5±0.55 | 1.8 |
| 3 | 30.0 | 30.0 | 31.2±0.75 | 1.5 |
| 4 | 40.0 | 40.0 | 40.5±0.40 | 1.2 |
| 5 | 50.0 | 50.0 | 50.2±0.65 | 1.6 |

^a Average of four replicate measurements

The detection limit was determined at 0.25 μM EP according to the definition of $Y_{LOD} = Y_B + 3\sigma$ [32].

Interference studies

The influence of various substances as potential interference compounds on the determination of EP under the optimum conditions with 50 μM EP at pH=10.0 was studied. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 5% for determination of EP. The results are given in Table 1, which shows that the peak current of EP is not significantly affected by all conventional cations, anions, and organic substances. Although ascorbic acid shows to have interference in the determination of EP, the tolerance limit allowed us to measure those amino acids in real samples such as urine. In addition, it can be minimized, if necessary, by using the ascorbic oxidase enzyme which exhibits high selectivity to the oxidation of ascorbic acid.

Determination of epinephrine in real samples

For more investigation, we study the applicability of the proposed sensor for the electrocatalytic determination of EP in real sample; we have selected urine sample. The results are given in Table 2. However, for more investigation, the epinephrine injection solution (specified content of EP is 1.00 mg mL⁻¹) was diluted to 250 mL with water; different capacity diluted solution was transferred into each of series of 10 mL volumetric flasks and diluted to the mark with

phosphate buffer. An aliquot of 10 mL of this test solution was placed in the electrochemical cell. The potentials were controlled between -0.1 and 0.25 V at the scan rate of 10 mV s^{-1} . I_{pa} was measured at the oxidation potential of EP. This procedure was repeated five times, and the relative standard deviation was obtained to be 2.8%. Different standard concentrations of EP were added to the diluted EP injection, and the recoveries were between 98.12% and 104.3% for five measurements. These results demonstrated the ability of *p*-CMMWCNTPE for voltammetric determination of EP in real samples with good recoveries of the spiked EP and good reproducibility.

Conclusion

A carbon paste electrode modified with *p*-chloranil, carbon nanotubes, and TiO_2 has been fabricated and used for electrocatalytic determination of EP. The results demonstrated that the electrooxidation of EP at the surface of *p*-CMMWCNTPE occurs at a potential about 171 mV less positive than bare carbon paste electrode. The modified electrode has been shown to be promising for EP detection with many desirable properties including good reproducibility, high sensitivity, excellent catalytic activity, low detection limit, and especially its antifouling properties towards EP and its oxidation products. Finally, this method was used for the determination of EP in real sample using standard addition method.

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