

## Simultaneous Determination of Isoproterenol, Acetaminophen and Folic Acid Using Nanostructured Electrochemical Sensor Based on Benzofuran Derivative and Carbon Nanotubes

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No presente artigo, o uso de pasta de eletrodo de carbono modificada por nanotubos de carbono e derivados de benzofurano (1-(4-(1,3-ditiolan-2-il)-6,7-dihidroxi-2-metil-6,7-dihydrobenzofuran-3-il) etanona) para a determinação de isoproterenol (IP) foi descrito. O coeficiente de transferência de carga,  $\alpha$ , e a constante de transferência de carga,  $k_s$ , para a transferência de elétrons entre o derivado de benzofurano e a pasta de eletrodo de carbono foram calculados como 0,52 e 1,04 s<sup>-1</sup>, respectivamente. O potencial anódico do IP diminui cerca de 256 mV com esse eletrodo modificado. Picos de corrente de voltametria de pulso diferencial (DPV) do IP, acetaminofeno (AC) e ácido fólico (FA) aumentam linearmente com concentrações na faixa de 0,05-2000  $\mu\text{mol L}^{-1}$ , 50,0-1200  $\mu\text{mol L}^{-1}$  e 67,0-1600  $\mu\text{mol L}^{-1}$ , respectivamente e os limites de detecção para IP, AC e FA foram 0,020  $\mu\text{mol L}^{-1}$ , 0,385  $\mu\text{mol L}^{-1}$  e 0,690  $\mu\text{mol L}^{-1}$ , respectivamente.

In the present paper, the use of carbon paste electrode modified by carbon nanotubes and benzofuran derivative (1-(4-(1,3-dithiolan-2-yl)-6,7-dihydroxy-2-methyl-6,7-dihydrobenzofuran-3-yl) ethanone) for the electrocatalytic determination of isoproterenol (IP) was described. The charge transfer coefficient,  $\alpha$ , and the charge transfer rate constant,  $k_s$ , for electron transfer between the benzofuran derivative and the carbon paste electrode were calculated as 0.52 and 1.04 s<sup>-1</sup>, respectively. The anodic overpotential of IP is decreased about 256 mV by this modified electrode. Differential pulse voltammetric (DPV) peak currents of IP, acetaminophen (AC) and folic acid (FA) increased linearly with their concentration in the ranges of 0.05-2000.0  $\mu\text{mol L}^{-1}$ , 50.0-1200.0  $\mu\text{mol L}^{-1}$  and 67.0-1600.0  $\mu\text{mol L}^{-1}$ , respectively and the detection limits for IP, AC and FA were 0.020  $\mu\text{mol L}^{-1}$ , 0.385  $\mu\text{mol L}^{-1}$  and 0.690  $\mu\text{mol L}^{-1}$ , respectively.

**Keywords:** carbon paste electrode, carbon nanotube, isoproterenol, acetaminophen, folic acid

### Introduction

Carbon nanotubes (CNTs), a new form of elementary carbon, are promising building blocks for nano science and nanotechnology because of their good mechanical strength, high surface area and interesting electronic properties.<sup>1,2</sup> We have successfully applied CNT modified electrodes to study and determination of many biological and organic molecules.<sup>3-5</sup>

Isoproterenol (IP), 4-[1-hydroxy-2-[(1-methylethyl)-amino]ethyl]-1, 2-benzenediol, is a catecholamine drug widely used for the hypertension and allergic emergencies,

bronchitis, status asthmatic, cardiac shock and heart attack.<sup>6</sup> Many methods have been developed to determine IP such as chromatography,<sup>7</sup> chemiluminescence,<sup>8</sup> spectrofluorimetry,<sup>9-11</sup> spectrophotometry<sup>12</sup> and electrochemical detection (for HPLC).<sup>13-15</sup> While these methods are often time consuming, complicated and expensive, electrochemical methods such as voltammetric and amperometric detections are simple, rapid, inexpensive and sensitive techniques for the determination of IP.<sup>16-21</sup>

Acetaminophen (AC), *N*-acetyl-*p*-aminophenol, is used in pain alleviation and bacterial fevers reducer. It is a suitable alternative for the patients who are sensitive to aspirin.<sup>22</sup> Also, AC is an electroactive molecule (AC contains hydroxyl and NH groups on its aromatic rings),

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and its electrochemical behavior has been studied widely. Many methods have been reported for the determination of AC including spectrophotometry,<sup>23</sup> liquid chromatography<sup>24</sup> and electrochemical methods.<sup>25-26</sup>

Folic acid (FA), (*N*-(4-[[2-amino-4-oxo-1,4-dihydropteridin-6-yl)], is a water-soluble B<sub>9</sub> vitamin that helps build healthy cells. Deficiency of FA is a common cause of anemia and it is thought to increase the likelihood of heart attack and stroke. There are many methods for the measurement of FA, including liquid chromatography,<sup>27,28</sup> capillary electrophoresis (CE),<sup>29</sup> spectrophotometric methods<sup>30</sup> and high-performance liquid chromatography (HPLC).<sup>31</sup> There are some electrochemical methods for the measurement of FA; because of FA is an electroactive component.<sup>32</sup> The detection limit, linear dynamic range, and sensitivity of the electrochemical methods to measure FA are comparable and even better than, those mentioned techniques.

To our knowledge, no study has been published so far reporting on the simultaneous determination of IP, AC and FA using 1-(4-(1,3-dithiolan-2-yl)-6,7-dihydroxy-2-methyl-6,7-dihydrobenzofuran-3-yl) ethanone (DDE) modified carbon paste electrode. So, in the present work, we described initially the preparation of a carbon paste electrode (CPE) modified with both DDE and carbon nanotubes as a new electrode in the electrocatalysis and determination of IP and then we evaluated the analytical performance of the modified electrode for simultaneous determination of IP, AC and FA. High sensitivity, selectivity and reproducibility of the voltammetric responses, and low detection limit, together with the ease of preparation make the proposed modified electrode very useful for accurate determination of IP, AC and FA contents in real samples.

## Experimental

### Apparatus and reagents

Voltammetric measurements were performed using a computerized potentiostat/galvanostat (SAMA 500, Iran). All electrochemical studies were performed at  $25 \pm 1$  °C with a three electrodes assembly including a saturated calomel electrode (SCE) as a reference electrode and a platinum wire as the counter electrode. The working electrode was a modified carbon paste electrode. A Metrohm 781 pH/ion meter was used for pH measurements. All solutions were freshly prepared with double-distilled water. IP, AC, FA, and reagents were analytical grade from Aldrich. Pure graphite fine powders (Merck) and paraffin oil (DC 350, Merck) were used as binding agents for the graphite pastes. Multiwall carbon nanotubes (purity more

than 95%) with o.d. between 5-20 nm, i.d. between 2-6 nm, and tube length 1-10  $\mu\text{m}$  were purchased from Plasma Chem. Before use, flasks and containers was soaked in 6 mol L<sup>-1</sup> HNO<sub>3</sub> for least 24 h, then rinsed with deionized water. Phosphate buffer solutions (0.1 mol L<sup>-1</sup>) were prepared from 0.1 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub>, and the pH was adjusted with 0.1 mol L<sup>-1</sup> H<sub>3</sub>PO<sub>4</sub> or NaOH.

### Synthesis of (1-(4-(1,3-dithiolan-2-yl)-6,7-dihydroxy-2-methyl-6,7-dihydrobenzofuran-3-yl) ethanone)

DDE was synthesized by electrosynthesis method and the manner described in our previous work.<sup>33</sup> Briefly, 80 mL of 0.15 mol L<sup>-1</sup> phosphate buffer (pH 7.0) in water:acetonitrile (85:15 volume ratio), containing 0.7 mmol of 4-(1,3-dithiolan-2-yl) benzene-1,2-diol and 0.7 mmol acetylacetone, was electrolyzed at controlled-potential in a divided cell equipped with a carbon anode (an assembly of four rods) and a large stainless steel gauze as cathode, at 0.35 vs. SCE. The electrolysis was terminated when the current decayed to 5% of its original value. The precipitated solid was collected by filtration and was washed several times with water.

### Oxidation of MWCNTs

Since the oxygen functionalities on the surface of multiwall carbon nanotubes (MWCNTs) improve their electrochemical properties, they were generated by treating MWCNTs with a mixture of concentrated H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> (molar ratio 3:1) following the method reported in the literature.<sup>34,35</sup> In a typical experiment, 75.0 mL of concentrated H<sub>2</sub>SO<sub>4</sub> (97%) and 25.0 mL of concentrated HNO<sub>3</sub> (65%) were mixed and added to 1.0 g of MWCNTs in a round-bottomed flask and heated under constant agitation at 50.0 °C for 8.0 h. It was allowed to cool down to room temperature after which an equal quantity of deionized water was added. It was filtered and the residue was washed several times with deionized water until neutral pH was attained. The residue was then filtered and freeze-dried.

### Preparation of the electrode

The DDE modified carbon paste electrode (DDECNPE) was prepared by mixing 0.4825 g of graphite powder, 0.0025 g of DDE, 0.7 mL of paraffin oil and 0.015 g of MWCNT with a mortar and pestle until a uniform paste was obtained. These amounts of materials were obtained by optimization. This paste was then packed into the end of a glass tube (ca. 10 cm long and 3.6 mm i.d.). A copper

wire inserted into the carbon paste provided an electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper. Also, unmodified carbon paste was constructed in the same way but without adding DDE and MWCNT to the mixture.

#### Preparation of real samples

Injection solution (0.10 mL, 0.20 mg mL<sup>-1</sup>) plus 10 mL of 0.1 mol L<sup>-1</sup> phosphate buffer solution (pH 7.0) were used for the analysis. The solution was transferred into the voltammetric cell to be analyzed without any further pretreatment. The standard addition method was used for the determination of isoproterenol (IP) in the samples.

## Results and Discussion

### Electrochemical properties of DDECNPE

The DDE is insoluble in aqueous solutions, and can be easily incorporated into the carbon paste without much concern of its leaching from the electrode surface. This fabrication process yields a stable, chemically-modified electrode. The electrochemical behavior of the DDECNPE was first studied using cyclic voltammetry (Figure 1). Experimental results show that well defined and reproducible anodic and cathodic peaks were obtained with  $E_{pa} = 0.150$  V vs. SCE,  $E_{pc} = 0.106$  V vs. SCE at a scan rate of 50 mV s<sup>-1</sup>. The half-wave potential ( $E_{1/2}$ ) was 0.128 V vs. SCE and  $\Delta E_p (= E_{pa} - E_{pc})$  was 0.044 V. The electrode process was *quasi-reversible*, with  $\Delta E_p$  greater than the (59/n) mV expected for a reversible system.

Cyclic voltammograms of the DDECNPE were recorded at different scan rates (from 10 to 900 mV s<sup>-1</sup>). Figure 1a illustrates that the anodic and cathodic peak currents ( $I_p$ ) were linearly dependent on  $v$  at scan rates of 10-900 mV s<sup>-1</sup>. A linear correlation was obtained between peak currents and the scan rate, indicating that the redox process is not controlled by diffusion. Figure 1c shows the anodic peak potentials,  $E_{pa}$ , as a function of the potential sweep rate. We found that for scan rates above 60 mV s<sup>-1</sup>, the values of  $E_p$  were proportional to the logarithm of the scan rate. Under these conditions, the  $k_s$  can be calculated according to the following equation:<sup>36</sup>

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log(RT / n_\alpha Fv) - \alpha(1 - \alpha) n_\alpha F \Delta E_p / 2.3RT \quad (1)$$

where  $n_\alpha$  represents the number of electrons involved in the rate-determining step and other symbols have their usual

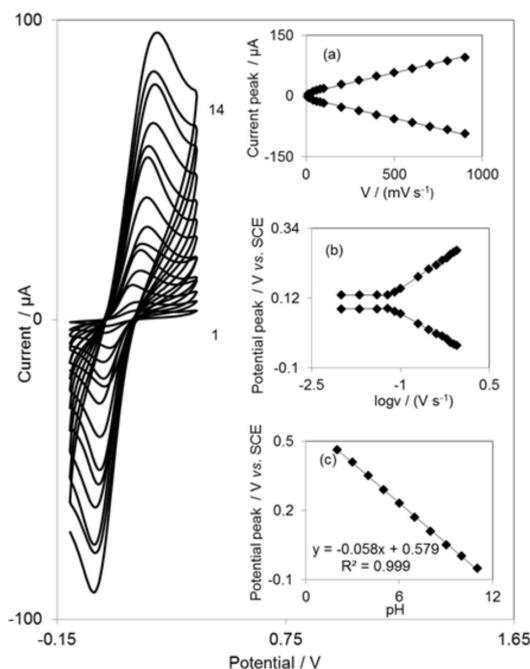
meanings. A mean value of  $k_s = 1.04$  s<sup>-1</sup> was evaluated from all the extracted experimental data applying equation 1. The slopes of Figure 1b plot can be used to extract the kinetic parameters cathodic transfer coefficients ( $\alpha_c$ ) and anodic transfer coefficients ( $\alpha_a$ ). The slope of the line segment is equal to  $-2.3RT / \alpha nF$  and  $2.3RT / (1 - \alpha) nF$  for the cathodic and anodic peaks, while the evaluated values for the anodic transfer coefficients ( $\alpha_a$ ) is 0.49.

An approximate estimate of the surface coverage of the electrode was made by adopting the method used by Sharp.<sup>37</sup> According to this method, the peak current is related to the surface concentration of the electroactive species,  $\Gamma$ , by the following equation:

$$I_p = n^2 F^2 A \Gamma v / 4RT \quad (2)$$

where  $n$  represents the number of electrons involved in the reaction,  $A$  is the surface area (0.096 cm<sup>2</sup>) of the electrode,  $\Gamma$  (mol cm<sup>-2</sup>) is the surface coverage, and the other symbols have their usual meanings. From the slope of the anodic peak currents *versus* the scan rate in Figure 1a, the calculated surface concentration is  $8.4 \times 10^{-11}$  mol cm<sup>-2</sup> for  $n = 2$ .

The electrochemistry of DDE molecule is generally pH dependent. Thus, the electrochemical behavior of DDECNPE was studied at different pHs using cyclic voltammetry (CV),

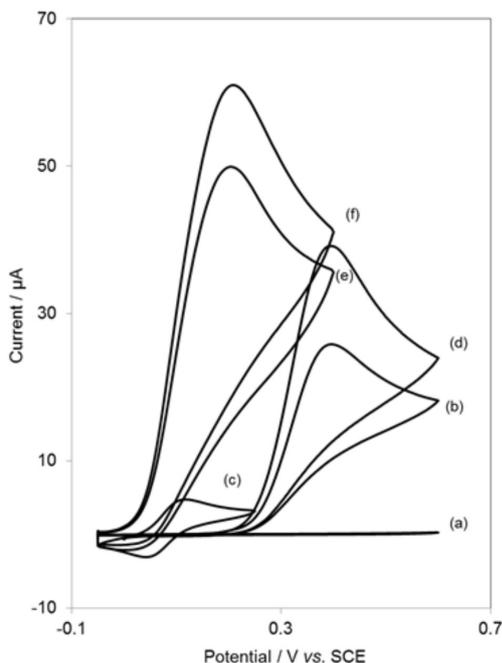


**Figure 1.** Cyclic voltammograms of DDECNPE in 0.1 mol L<sup>-1</sup> phosphate buffer (pH 7.0), at various scan rates: the numbers 1-14 correspond to 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600, 700, 800 and 900 mV s<sup>-1</sup> scan rates, respectively. Insets: (a) Variations of peak currents *versus* scan rates; (b) variation of peak potentials *versus* the logarithm of the scan rate; (c) plot of  $E_p'$  of DDECNPE *versus* pH.

see Figure 1c. Since one straight line was obtained with a slope value of  $-58$  mV per pH in the pH ranges of 2.0-11.0, there is a transfer of two electrons and two protons in the redox reaction of DDE in the pH range of 2.0-11.0.<sup>38</sup>

#### Electrocatalytic oxidation of IP at a DDECNPE

Figure 2 depicts the CV responses for the electrochemical oxidation of  $0.5 \text{ mmol L}^{-1}$  IP at unmodified CPE (curve b), CNPE (curve d), DDECPE (curve e) and DDECNPE (curve f). While the anodic peak potentials for IP oxidation at the CNPE, and unmodified CPE are 350 and 406 mV, respectively, the corresponding potential at DDECNPE and DDECPE is ca. 150 mV. These results indicate that the peak potential for IP oxidation at the DDECNPE and DDECPE electrodes shifts by ca. 200 and 256 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, DDECNPE shows much higher anodic peak current for the oxidation of IP compared to DDECPE, indicating that the combination of CNTs and the mediator (DDE) has significantly improved the performance of the electrode toward IP oxidation. In fact, DDECNPE in the absence of IP exhibited a well-behaved redox reaction (Figure 2, curve c) in  $0.1 \text{ mol L}^{-1}$  phosphate buffer (pH 7.0), without IP in solution. However, there was a drastic increase in the anodic peak current in the presence of  $0.5 \text{ mmol L}^{-1}$  IP (curve f), which can be related to the



**Figure 2.** Cyclic voltammograms of (a) CPE in  $0.1 \text{ mol L}^{-1}$  phosphate buffer solution (pH 7.0) at scan rate  $20 \text{ mV s}^{-1}$  and (b) as (a)  $0.50 \text{ mmol L}^{-1}$  IP; (c) as (a) and (d) as (b) at the surface of DDECNPE and DDECPE respectively. Also, (e) and (f) as (b) at the surface of DDECPE and DDECNPE, respectively.

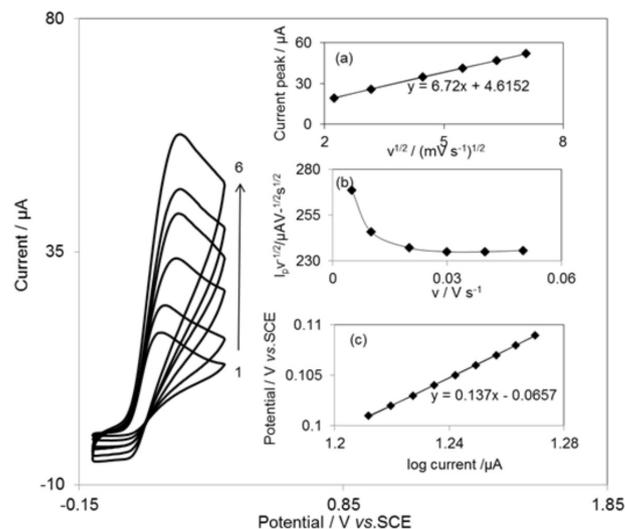
strong electrocatalytic effect of the DDECNPE toward this compound.<sup>38</sup>

The scan rate dependence of cyclic voltammograms of the DDECPE, in  $0.1 \text{ mol L}^{-1}$  phosphate buffer solution containing  $0.5 \text{ mmol L}^{-1}$  IP, is presented in Figure 3. Inset exhibits that a plot of the catalytic peak current *versus* the square root of the sweep rate is linear, showing that at sufficient overpotential, the reaction is diffusion-limited. A plot of the sweep rate normalized current ( $I_p/v^{1/2}$ ) *versus* sweep rate inset b, exhibits the characteristic shape, a kind of an  $\text{EC}_{\text{cat}}$  process.

The number of electrons in the overall reaction can also be acquired from the slope of the  $I_p$  *versus*  $v^{1/2}$  plot Figure 3, inset a. Using the slope of this plot and according to the following equation for a totally irreversible diffusion controlled process<sup>39</sup>

$$I_p = 3.01 \times 10^5 n [(1 - \alpha) n \alpha]^{1/2} A C_b D^{1/2} v^{1/2} \quad (3)$$

it is estimated that the total number of electrons involved in the anodic oxidation of IP is  $n = 2$ . A Tafel plot is a useful device for evaluating the kinetic parameters. Inset c of Figure 3 shows the Tafel plot, drawn by using the data derived from the rising part of the current-voltage curve at a scan rate of  $20 \text{ mV s}^{-1}$ . The number of electrons involved in the rate-determining step ( $n_\alpha$ ) can be estimated from the slope of the Tafel plot.<sup>38</sup> A slope  $0.137 \text{ V}$  is acquired indicating a one electron transfer to be rate limiting step assuming a transfer coefficient of  $\alpha = 0.57$ .



**Figure 3.** Cyclic voltammograms of DDECNPE in  $0.1 \text{ mol L}^{-1}$  phosphate buffer (pH 7.0) containing  $0.25 \text{ mmol L}^{-1}$  IP at different scan rates. The numbers 1-6 correspond to 5, 10, 20, 30, 40 and  $50 \text{ mV s}^{-1}$  scan rates, respectively. Insets: (a) variation of the electrocatalytic currents *versus* the square root of scan rate; (b) variation of the scan rate normalized current ( $I_p/v^{1/2}$ ) with scan rate and (c) Tafel plot, derived from the current potential curve, recorded at the scan rate of  $20 \text{ mV s}^{-1}$ .

### Chronoamperometric measurements

The catalytic oxidation of IP by DDECNPE was examined by chronoamperometry. Chronoamperograms acquired at a potential step of 250 mV are represented in Figure 4. In chronoamperometric studies, we have determined the diffusion coefficient of IP for a DDECNPE. For IP with a diffusion coefficient of  $D$  in  $\text{cm}^2 \text{s}^{-1}$ , the current for the electrochemical reaction (at a mass transport restricted rate) is described by the Cottrell equation.<sup>38</sup>

$$I = nFAD^{1/2}C_b / \pi^{-1/2} t^{-1/2} \quad (4)$$

where  $C_b$  is in  $\text{mol cm}^{-3}$  and  $D$  in  $\text{cm}^2 \text{s}^{-1}$  are the bulk concentration and the diffusion coefficient, respectively. The experimental plots of  $I$  versus  $t^{-1/2}$  for different concentrations of IP are depicted in Figure 4a. The slopes of the resulting straight lines were then plotted versus the IP concentration Figure 4b. Based on the Cottrell equation, using the slope of the linear relation in Figure 4b, we estimate the diffusion coefficient of IP to be  $D = 2.42 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .

We have also used the chronoamperometric method of Galus to determine the catalytic rate constant,  $k$  in  $\text{mol}^{-1} \text{ L s}^{-1}$ , for the reaction between IP and the DDECNPE.<sup>40</sup>

$$I_c/I_L = \gamma^{1/2} [\pi^{1/2} \text{erf}(\gamma^{1/2}) + \exp(-\gamma) \gamma^{-1/2}] \quad (5)$$

where  $I_c$  is the catalytic current of IP at the DDECNPE,  $I_L$  the limited current in the absence of IP and  $\gamma = kC_b t$

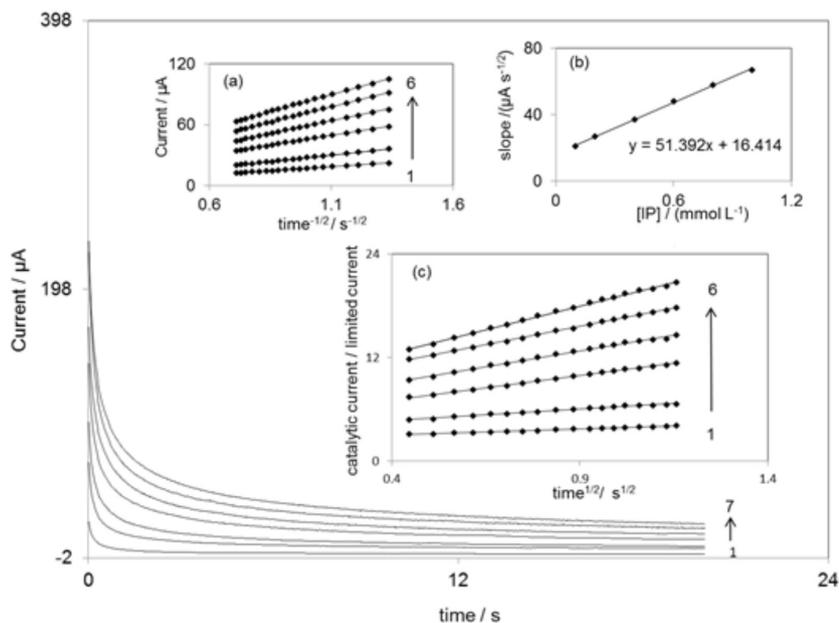
( $C_b$  is the bulk concentration of IP) is the argument of the error function. In the cases where  $\gamma$  exceeds 2, the error function is almost equal to 1 and the above equation can be reduced to:

$$I_c/I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \quad (6)$$

where  $t$  in  $s$  is the time elapsed. The above equation can be used to calculate the rate constant ( $k$  in  $\text{mol}^{-1} \text{ L s}^{-1}$ ) of the catalytic process. Based on the slope of the  $I_c/I_L$  versus  $t^{1/2}$  plot,  $k$  can be acquired for a given IP concentration. Such plots obtained from the chronoamperograms in Figure 4 are shown in inset c. From the values of the slopes, the average value of  $k$  was found to be  $k = 2.336 \times 10^4 \text{ mol}^{-1} \text{ L s}^{-1}$ . The value of  $k$  explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of IP at the surface of DDECNPE.

### Calibration plot and limit of detection

Since differential pulse voltammetry (DPV) has a much higher current sensitivity and suitable resolution from cyclic voltammetry, it was used to estimate the limit of detection of IP. Voltammograms clearly show that the plot of peak current versus IP concentration is constituted of two linear segments with different slopes (equations of  $I$  ( $\mu\text{A}$ ) =  $0.430 C_{IP}$  ( $\mu\text{mol L}^{-1}$ ) +  $17.1$  ( $\mu\text{A}$ ) for the first segment and  $I$  ( $\mu\text{A}$ ) =  $0.025 C_{IP}$  ( $\mu\text{mol L}^{-1}$ ) +  $36.3$  ( $\mu\text{A}$ ) for the second segment) corresponding to two different ranges of substrate concentration,  $0.050$  to  $50.0 \mu\text{mol L}^{-1}$



**Figure 4.** Chronoamperograms obtained at DDECNPE in  $0.1 \text{ mol L}^{-1}$  phosphate buffer solution (pH 7.0) for concentration of IP: 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and  $1.0 \text{ mmol L}^{-1}$ . Insets: (a) plots of current vs.  $\text{time}^{-1/2}$  obtained from chronoamperograms; (b) plot of the slope of the straight lines against the IP concentration and (c) dependence of  $I_c/I_L$  on  $\text{time}^{1/2}$  derived from the data of chronoamperograms.

**Table 1.** Comparison of some electrochemical procedures used in the determination of IP

Electrode	Modifier	pH	Linear range / ( $\mu\text{mol L}^{-1}$ )	Detection limit / ( $\mu\text{mol L}^{-1}$ )	Peak potential shift / mV	Ref.
GCE	DDF <sup>a</sup>	7.0	0.1-1300	0.028	250	16
CPE	5ADB <sup>b</sup>	7.0	0.4-900	0.2	215	17
CPE	<i>P</i> -chloranil	10.5	0.015-100	0.009	250	41
GCE	Poly(1-methylpyrrole)-DNA	4.0	2.0-50	0.16	334	42
CPE	<sup>c</sup> DDECNPE	7.0	0.050-2000	0.02	256	This work

<sup>a</sup>9-(1,3-dithiolan-2-yl)-6,7-dihydroxy-3,3-dimethyl-3,4-dihydrodibenzo[b,d]furan-1(2H)-one; <sup>b</sup>5-amino-3',4'-dimethyl-biphenyl-2-ol; <sup>c</sup>1-(4-(1,3-dithiolan-2-yl)-6,7-dihydroxy-2-methyl-6,7-dihydrobenzofuran-3-yl) ethanone of IP concentration; (b) plot of the peak currents as a function of AC concentration and (c) plot of the peak currents as a function of FA concentration.

for the first linear segment and 50.0 to 2000.0  $\mu\text{mol L}^{-1}$  for the second linear segment. The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation. From the analysis of data, we estimated that the lower limit of detection of IP is approximately 0.020  $\mu\text{mol L}^{-1}$  based on the following equation:

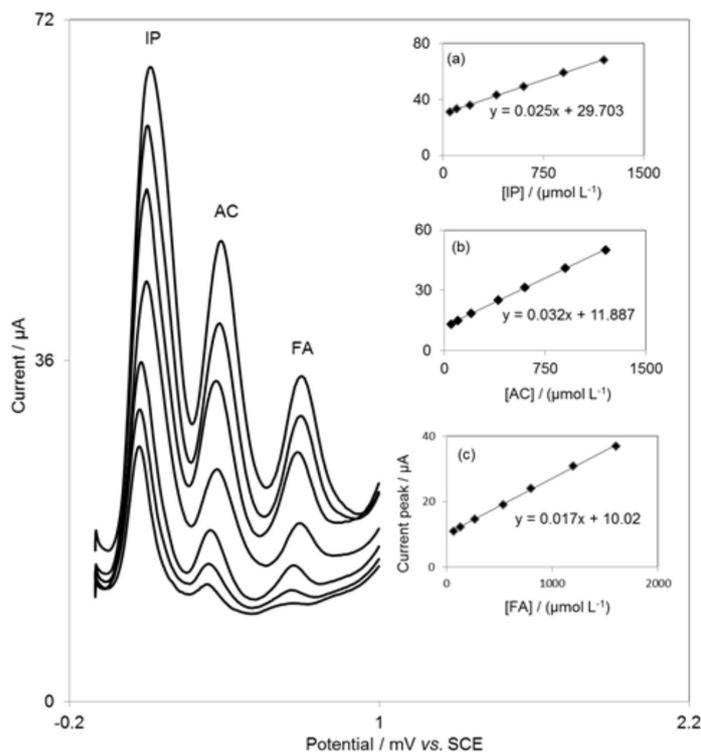
$DL = 3Sb/m$  (where  $Sb$  is the standard deviation of the blank and  $m$  is the slope of the calibration plot).

Table 1 shows some analytical parameters such as detection limit and linear range for determination of IP by the proposed electrode in comparison with some other electrochemical procedures. According to the Table 1,

detection limit, linear range and pH that used at this work are better than other works.<sup>16,17,41,42</sup>

#### Simultaneous determination of IP, AC and FA

DDECNPE was used for the simultaneous determination of IP, AC and FA by simultaneously changing their concentrations in the solution. Figure 5 insets a, b and c show the dependence of DPV peak currents on the concentration of IP, AC and FA at the DDECNPE, respectively. Differential pulse voltammetry results show three well-distinguished anodic peaks at potentials of



**Figure 5.** Differential pulse voltammograms of DDECNPE in 0.1 mol L<sup>-1</sup> phosphate buffer solution (pH 7.0) containing different concentrations of IP, AC and FA. (from inner to outer) mixed solutions of 50 + 50 + 67; 100 + 100 + 133; 200 + 200 + 267; 400 + 400 + 533; 600 + 600 + 800; 900 + 900 + 1200 and 1200 + 1200 + 1600  $\mu\text{mol L}^{-1}$ , respectively, in which the first value is concentration of IP in  $\mu\text{mol L}^{-1}$ , the second value is concentration of AC in  $\mu\text{mol L}^{-1}$  and the third value is concentration of FA in  $\mu\text{mol L}^{-1}$ . Insets: (a) plot of the peak currents as a function of IP concentration; (b) plot of the peak currents as a function of AC concentration and (c) plot of the peak currents as a function of FA concentration.

**Table 2.** Determination of IP, AC and FA in synthetic solutions using DDECNPE by standard addition method

Number	Synthetic solution / ( $\mu\text{mol L}^{-1}$ )			Found <sup>a</sup> / ( $\mu\text{mol L}^{-1}$ )			Recovery / %		
	IP	AC	FA	IP	AC	FA	IP	AC	FA
1	10	0	0	10.3	–	–	103.0	–	–
2	20	0	0	20.1	–	–	100.5	–	–
3	0	10	0	–	9.7	–	–	97.0	–
4	0	20	0	–	20.6	–	–	103.0	–
5	0	0	10	–	–	9.6	–	–	96.0
6	0	0	20	–	–	21.0	–	–	105.0
7	10	10	10	9.7	10.2	10.3	97.0	102.0	103.0
8	20	20	20	20.2	20.4	19.3	101.0	102.0	96.5

<sup>a</sup>Mean value for five replicate measurements.

0.101, 0.385 and 0.690 V indicating that the simultaneous determination of IP, AC and FA is possible at the DDECNPE.

Also, using DDECNPE as the working electrode, we obtained the linear range simulation of the calibration curve for AC and FA as 50.0-1200 and 67.0-1600  $\mu\text{mol L}^{-1}$  and the detection limit (3s) 0.385 and 0.690  $\mu\text{mol L}^{-1}$ , respectively.

#### Real sample analysis

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of IP in ampoule purchased from local sources. The concentration of IP was carried out by standard addition method in order to prevent any matrix effect. The amount of unknown IP in the ampoule can be detected by extrapolating the plot. The average amount of IP in the injection was found to be  $0.18 \pm 0.01$  mg, which is in good agreement with the accepted value (0.20 mg). Also, for investigation of the applicability of DDECNPE for simultaneous determination of IP, AC and FA in real sample, this electrode was applied to mixture solutions. Table 2 shows the recovery percent of IP, AC and FA in synthetic solutions using DDECNPE by the standard addition method.

#### Interference studies

The influence of various species interfering with the determination of IP 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 IP. According to the results,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , *l*-lysine, glucose, tryptophan, *n*-acetyl cysteine, gemfibrozil, atenolol, pohenyl propanl and uric acid did not show interference in the determination of IP, but, 0.5 mmol  $\text{L}^{-1}$  of dopamine and ascorbic acid showed interference on determination of 0.5 mmol  $\text{L}^{-1}$  IP.

## Conclusion

The DDECNPE was prepared and used for the investigation of the electrochemical behaviour of IP. The DDECNPE showed excellent electrocatalytic activity for the oxidation of IP. The DPV currents of IP at DDECNPE increased linearly with the IP concentration in the range from 0.050 to 2000  $\mu\text{mol L}^{-1}$  with a detection limit of 0.020  $\mu\text{mol L}^{-1}$ .

The DDECNPE exhibits high electrocatalytic activity for oxidation of IP, AC and FA. The high current sensitivity, low detection limit, the ease of preparation, high repeatability and high selectivity of the DDECNPE for the detection of IP prove its potential sensing applications. The results show that the oxidation of IP is catalyzed at pH 7.0.

The peak potential of IP is shifted by 256 mV at the surface of the DDECNPE. Also, DDECNPE was used for determination of IP, AC and FA in some real samples.

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