

# Electrochemical determination of captopril in the presence of acetaminophen, tryptophan, folic acid, and L-cysteine at the surface of modified carbon nanotube paste electrode

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**Abstract** A novel carbon paste electrode (CPE) modified with 2,2'-[1,7-heptanediy]bis(nitrilomethylidene)-bis(4-hydroxyphenol) (DHB) and carbon nanotubes (CNTs) was prepared. At first, the redox properties of the modified electrode were studied by cyclic voltammetry (CV). Then, the modified electrode was used as an electrochemical sensor for oxidation of captopril (CAP). Under the optimum pH of 7.0, the overpotential of CAP oxidation decreases about 120 mV at modified electrode than unmodified CPE. Differential pulse voltammetry (DPV) of CAP at the electrochemical sensor exhibited two linear dynamic ranges (7.0–100.0 and 100.0–2,500.0  $\mu\text{M}$ ) with a detection limit ( $3\sigma$ ) of 2.43  $\mu\text{M}$ . DPV was used for determination of CAP in the presence of acetaminophen (AC) and tryptophan (Trp), CAP in the presence of folic acid (FA), and CAP in the presence of L-cysteine (L-Cys) by the electrochemical sensor. The proposed electrochemical sensor was used for the determination of these substances in real sample.

**Keywords** Modified electrode · Carbon nanotube ·  
Electrocatalysis · Captopril

## Introduction

Among the application of nanomaterials, carbon nanotubes (CNTs) have become the subject of intense investigation since

their discovery in 1991 by Iijima [1]. The electrochemical methods using chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for the detection of the trace amounts of biologically important compounds [2–5]. One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential respect to unmodified electrodes. Recently, there has been an increasing demand for more sensitive and simple analytical methods. Voltammetric techniques are very useful and popular for trace analysis, since these techniques are compact, efficient, and sensitive [6–11]. However, some authors have emphasized the instability of the attached materials on the electrodes as a problem arising in the utilization of chemically modified electrodes. It seems that the incorporation of electrocatalysts into the electrode matrix can, even partly, help to solve these problems, and carbon paste electrodes (CPEs) spiked with catalyst may be suitable for this purpose. The ease and speed of preparation and obtaining a new reproducible surface, the low residual current, porous surface, and low cost are some advantages of chemically modified carbon paste electrodes [12].

Captopril (CAP) with the chemical name of (2S)-1-[(2S)-2-methyl-3-sulfanylpropanoyl] pyrrolidine-2-carboxylic acid is an angiotensin-converting enzyme inhibitor (ACE inhibitor) and has been widely used as antihypertensive drug. CAP is used to treat high blood pressure and heart failure [13]. It decreases certain chemicals that tighten the blood vessels, so blood flows more smoothly and the heart can pump blood more efficiently. Serious toxicity has occurred primarily when CAP was given in high doses to patients with collagen vascular disease or renal insufficiency. Minor toxic effects which are seen include altered sense of taste, allergic skin rashes, and drug fever [14]. Various instrumental methods have been developed for the determination of captopril including HPLC [15], spectrophotometry [16], and chemiluminescence

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methods [17]. Nevertheless, these methods often have diverse disadvantages such as high cost, low selectivity, the use of organic solvents, complex sample preparation procedures, or long analysis time. In contrast, electrochemical methods can offer several advantages, for example, an inexpensive and simple analytical method with remarkable detection sensitivity, reproducibility, and ease of miniaturization [18–24]; they have been used in a wide range of important applications in clinical, industrial, environmental, and agricultural analyses [25–39]. Also, they are superior for use in the analytical determination of captopril [40–43].

Acetaminophen (AC) is categorized as analgesics (pain relievers) and antipyretics (fever reducers). When used appropriately, side effects are rare. The most serious side effect is liver damage due to large doses, chronic use, or concomitant use with alcohol or other drugs. AC is electroactive, and can be oxidized under certain conditions. Therefore, different electrochemical methods using various modified electrodes have also been proposed for the determination of AC [4, 44].

Tryptophan (Trp) is an essential amino acid, meaning that humans must consume it in their diet to survive. This compound is a precursor for the important biological molecules serotonin (a neurotransmitter), melatonin (a neurohormone), and niacin. It has also been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. When improperly metabolized, it creates a byproduct in the brain that is toxic, causing hallucination and delusions. Due to its low abundance in vegetables, this compound is sometimes added to staple food products and pharmaceutical formulas [45]. The electroanalytical methods, with respect to their sensitivity, accuracy, lower cost, and simplicity, have been more attractive in recent years for Trp determination [46, 47].

L-cysteine (L-Cys) is an important thio-containing amino acid involved in a variety of important cellular functions, such as protein synthesis, detoxification, and metabolism [48]. It has been also used as a radioprotective agent, cancer indicator, and is implicated in a number of pathological conditions, including Alzheimer's and Parkinson's diseases as well as autoimmune deficiency syndrome [49]. On the other hand, a deficiency of L-Cys causes many diseases, such as slowed growth in children, depigmentation of hair, edema, lethargy, liver damage, loss of muscle and fat, skin lesion, and weakness. Therefore, a sensitive and selective assessment of L-Cys in biological matrices and pharmaceutical preparations is highly demanded. The responses to thiol oxidation at unmodified electrodes usually suffer from large overpotentials or sluggish electrochemical responses [50]. The chemical modification of inert substrate electrodes with mediators offers significant advantages in the design and development of L-Cys electrochemical sensors. Therefore, biochemists and electroanalytical chemists have shown great interest in the area of modified electrodes to overcome these problems, and various modified electrodes have been constructed for this purpose [51].

Similarly, folic acid (FA) is an important component of the hematopoietic system and is the coenzyme that controls the generation of ferroheme. Lack of FA gives rise to gigantocytic anemia and is associated with leucopenia, devolution of mentality, and psychosis. Determination of FA is often required in pharmaceutical, clinical, and food samples [52].

According to the above points, we used new carbon paste electrode modified with 2,2'-[1,7-heptanediy]bis(nitrilomethylidene)]-bis(4-hydroxyphenol) (DHB) and CNTs and investigate its performance for the electrocatalytic determination of CAP in the presence of AC and Trp, CAP in the presence of FA, and CAP in the presence of L-Cys. The experimental results indicate that modified electrode offers several advantages such as good stability, fast response, low detection limit, large dynamic range, good selectivity, and high apparent charge transfer rate constant. Utilizing the developed method, determination of these compounds has been carried out in human blood serum samples.

## Experimental

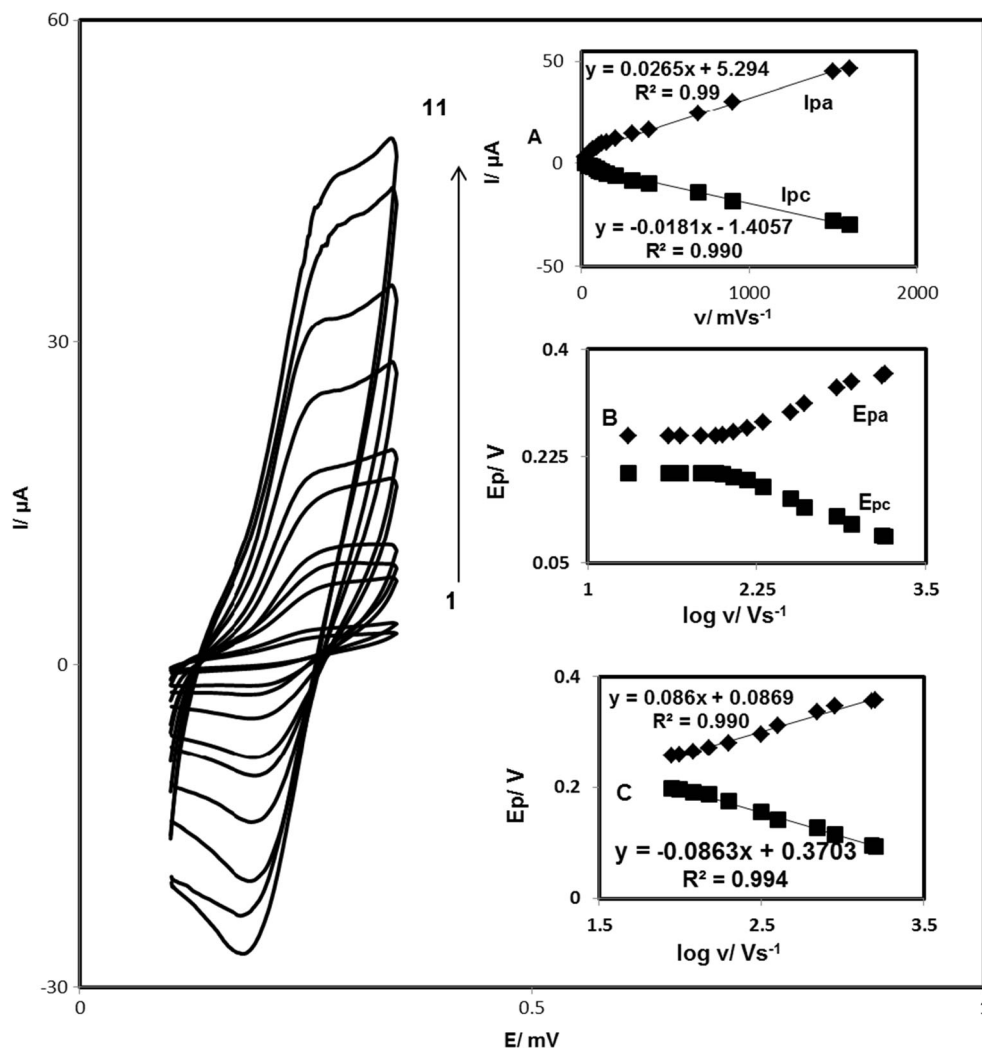
### Apparatus and chemicals

Electrochemical measurements were performed with a potentiostat/galvanostat (SAMA 500, electroanalyzer, system, IR. Iran). All experiments were carried out in a three-electrode cell at room temperature with DHB/CNT/CPE, SCE electrode, and a platinum wire as the working, reference, and counter electrodes, respectively (reference and auxiliary electrodes from AZAR electrode, Iran). All solutions were freshly prepared with doubly-distilled water. CAP, AC, Trp, FA, L-Cys, and all other reagents were analytical grade from Sigma-Aldrich. The graphite powder and paraffin oil ( $DC\ 0, 0.88\ g\ cm^{-3}$ ), both from Merck (Darmstadt, Germany), were used as received. Multiwalled carbon nanotubes (purity more than 95 %) with o.d. between 5 and 20 nm, i.d. between 2 and 6 nm, and tube length 1–10  $\mu m$  were purchased from plasma Chem (Germany). Phosphate buffers were prepared from orthophosphoric acid, and its salts and pH values were measured using a Metrohm 691 pH meter.

### A typical procedure for synthesis of 2,2'-[1,7-heptanediy]bis(nitrilomethylidene)]-bis(4-hydroxyphenol)

To a mixture of 2,5-dihydroxybenzaldehyde (0.35 g, 2.5 mmol) in MeOH was added 1,7-diaminoheptane (0.18 g, 1.4 mmol) and stirring for 30 min. The progress of the reaction was monitored by TLC. After the reaction completion, yellow solid product was filtered off and washed with cold MeOH, and pure Schiff base, 2,2'-[1,7-heptanediy] bis(nitrilomethylidene)]-bis(4-hydroxyphenol) was obtained in 90 % yield, m.p.= 215–217 °C. The Schiff base product was identified by physical and spectroscopic data as follows:

**Fig. 1** CVs of a DHB/CNT/CPE in 0.1 M phosphate buffer solution of pH 7.0 at various scan rates, the numbers 1–11 correspond to 20, 40, 100, 120, 150, 300, 400, 700, 900, 1,500, and 1,600  $\text{mV s}^{-1}$ ; **A** plot of  $E_{\text{pa}}$  versus  $\log v$ , **B** plot of  $E_{\text{p}}$  versus  $\log v$ , and **C** plot of  $I_{\text{p}}$  versus  $v$



2,2'-[1,7-heptanediyl-bis(nitriolomethylidene)]-bis(4-hydroxyphenol): yellow solid; yield: 90 %; M.p: 215–217 °C. Anal. Calcd.: C, 68.1; H, 7.02; N, 7.6. Found: C, 68.1; H, 6.9; N, 7.4. IR (KBr)/ $\nu(\text{cm}^{-1})$ : 3,373–3,500 (s, br, 2OH), 1,646 (s, C=N), 1,503, 1,457 (Ar), 1,239 (s, C-O).  $^1\text{H}$  NMR (400 MHz/DMSO- $d_6$ )/ $\delta$  ppm: 12.7 (br, 2OH, intramolecular hydrogen bonding), 8.90 (br, 2OH), 8.42 (s, 2CH Imine), 6.78 (d, 2H, Ar,  $J=4$  Hz), 6.76 (dd, 2H, Ar,  $J_1=8.2$  Hz,  $J_2=2.2$  Hz), 6.69 (d, 2H, Ar,  $J=8.4$  Hz), 3.54 (t, 4H, 2CH<sub>2</sub>,  $J=12.8$ Hz), 1.58 (m, 8H, 4CH<sub>2</sub>), 1.33 (m, 2H, CH<sub>2</sub>).  $^{13}\text{C}$  NMR(100 MHz/DMSO- $d_6$ )/ $\delta$  ppm: 165.77, 153.62, 149.70, 120.12, 119.01, 117.25, 116.90, 59.02, 30.83, 28.88, 27.03. MS:  $m/z=370$  ( $\text{M}^+$ , 4), 235 (6), 220 (22), 206 (28), 193 (90), 179 (32), 150 (68), 137 (92), 126(100). UV/ $\lambda_{\text{max}}$ : 340 (s), 240 (w).

#### Preparation of the electrode

The DHB/CNT/CPE were prepared by hand mixing 0.01 g of DHB with 0.96 g graphite powder and 0.03 g carbon nanotube particles with a mortar and pestle, Then ~0.7 mL of paraffin

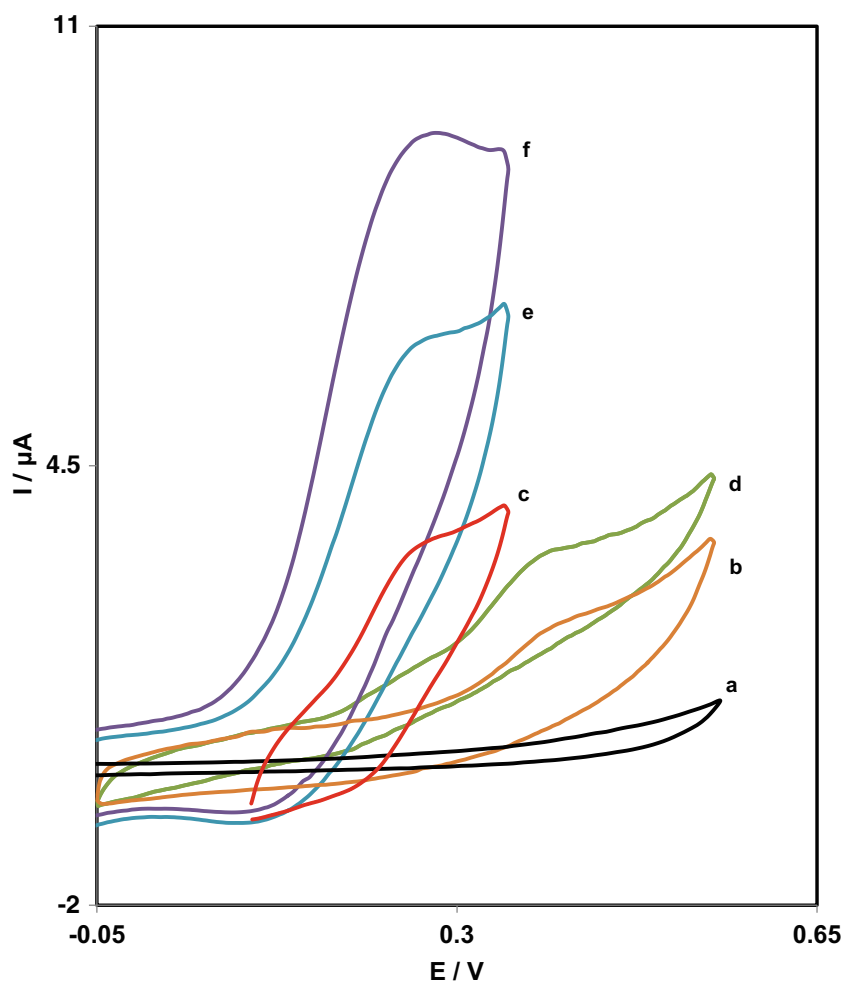
was added to the above mixture and mixed for 20 min until a uniformly wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.7 mm i.d. and 10 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste. Out of the tube and polishing with a weighing paper for comparison, modified CPE electrode (DHB/CPE) without CNTs, CNT paste electrode (CNT/CPE) without DHB, and unmodified CPE in the absence of both DHB and CNT were also prepared in the same way.

## Results and discussion

### Chemistry of DHB/CNT/CPE

One of the advantages of DHB as a modifier is its insolubility in aqueous media. Therefore, we prepared DHB/CNT/CPE

**Fig. 2** CVs of *a* unmodified CPE in 0.1 M phosphate buffer solution (pH 7.0) at scan rate of  $40 \text{ mV s}^{-1}$ ; *b* as (a) +0.5 mM CAP; *c* as (a) at the surface of DHB/CNT/CPE; *d* as (b) at the surface of CNT/CPE; *e* as (b) at the surface of DHB/CPE; and *f* as (b) at the surface of DHB/CNT/CPE



and studied its electrochemical properties in phosphate buffer solution (pH 7.0) using CV (Fig. 1). Since the DHB is insoluble in aqueous solution, we investigated its electrochemical behavior by carbon paste electrode. Experimental results showed reproducible, well-defined, anodic, and cathodic peaks with  $E_{pa}=0.283 \text{ V}$  and  $E_{pc}=0.182$  versus SCE. The observed peak separation potential,  $\Delta E_p=(E_{pa}-E_{pc})$  of 101 mV, was greater than the value of  $59/n \text{ mV}$  expected for a reversible system [53], suggesting that the redox couple of DHB in DHB/CNT/CPE has a quasi-reversible behavior in aqueous medium. The effect of the potential scan rate ( $\nu$ ) on electrochemical properties of the DHB/CNT/CPE was also studied by CV. Plots of the both anodic and cathodic peak currents ( $I_p$ ) were linearly dependent on  $\nu$  in the range of 20–1,600  $\text{mV s}^{-1}$  (Fig. 1a), indicating that the redox process of DHB at the modified electrode is diffusionless in nature [53].

The apparent charge transfer rate constant,  $k_s$ , and the transfer coefficient,  $\alpha$ , of a surface-confined redox couple can be evaluated from CV experiments by using the variation of anodic and cathodic peak potentials with logarithm of scan rate, according to the procedure of Laviron (1979). Figure 1b, c shows the variations of peak potentials ( $E_p$ ) as a

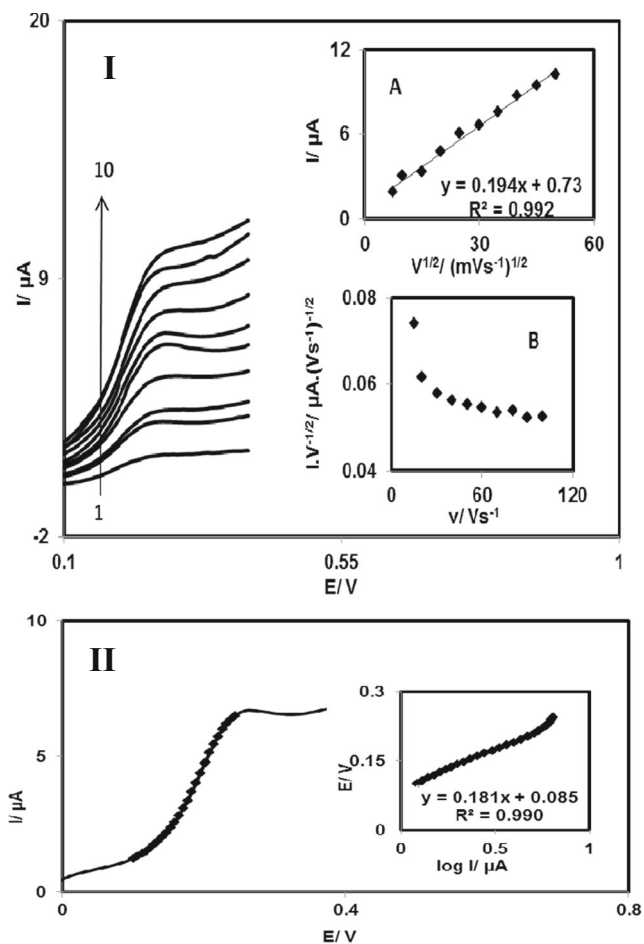
function of the logarithm of the potential scan rate at scan rates and high scan rates, respectively. As can be seen at high scan rates, these plots become linear. The slopes of these linear segments in Fig. 1c can be used to extract the kinetic parameters  $\alpha_c$  and  $\alpha_a$  (cathodic and anodic transfer coefficients, respectively). The slope of the linear segments are equal to  $-2.303RT/\alpha nF$  and  $2.303RT/(1-\alpha)nF$  for the cathodic and anodic peaks, respectively. The evaluated value for the  $\alpha_a$  is 0.32.

The Laviron equation can be used to gain the electron transfer rate constant for the case of surface-confined electroactive species with a concentration small enough [54].

$$\text{For } E_{pa}-E_{pc} = \Delta E_p > 200/n \text{ mV} : \\ \log(k_s/s^{-1}) = \alpha \log(1-\alpha) \log \alpha - \log(RT/nf\nu) / \quad (1)$$

$$s - \alpha(1-\alpha) n \Delta E_p / 2.3 RT$$

So, the value of apparent charge transfer rate constant ( $k_s$ )  $5.70 \text{ s}^{-1}$  were calculated for the DHB/CNT/CPE at 0.1 M phosphate buffer.



**Fig. 3** I Linear sweep voltammetry of a DBH in 0.1 M phosphate buffer (pH 7.0) containing 0.5 mM CAP at different scan rates; the numbers 1–10 correspond to 5, 15, 20, 30, 40, 50, 60, 70, 80, and 90 mV s<sup>-1</sup> scan rates, Insets: A variation of the electrocatalytic currents versus the square root of scan rate and B variation of the scan rate normalized current ( $I_p/v^{1/2}$ ) versus scan rate; II Tafel plot derived from the rising part of voltammogram recorded at a scan rate of 20 mV s<sup>-1</sup>

An approximate estimate of the surface coverage of the electrode,  $\Gamma$ , was made by adopting the method used by Sharp et al. [55] in Eq. (2):

$$I_p = n^2 F^2 A \Gamma^2 v / 4RT \tag{2}$$

According to this method, the peak current is related to the surface concentration of electroactive species. Where  $n$  shows the number of electrons involved in reaction,  $A$  is the surface area (0.1 cm<sup>2</sup>), and  $\Gamma$  obtained  $7.0 \times 10^{-8}$  mol cm<sup>-2</sup> for  $n=2$ .

The electrochemical behavior of DHB/CNT/CPE was studied at different pHs using CV. It was observed that the anodic peak potentials of DHB at DHB/CNT/CPE shift to negative values with increasing pH. The results showed that unit over a pH range from 4 to 9 the slope ( $E^0/\text{pH}$ ) is 47.0 mv/

pH, which is very close to the predicted Nernstian value of 59.2 mv for two electrons two protons [53].

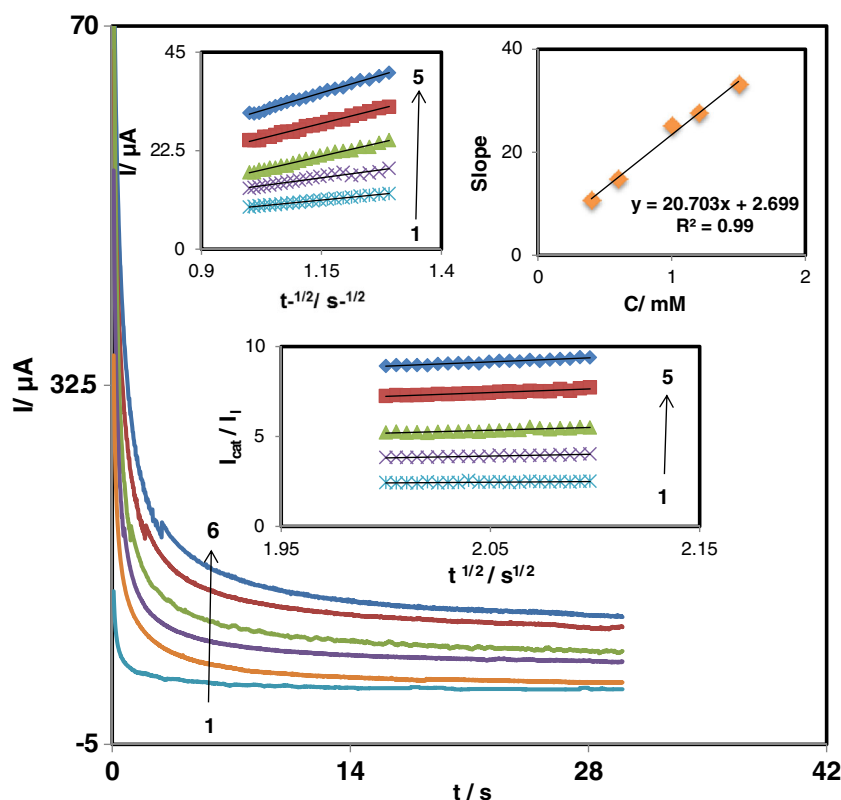
### Electrocatalytic oxidation of CAP at a DHB/CNT/CPE

Figure 2 shows the CVs responses for the electrochemical oxidation of 0.5 mM CAP at the unmodified CPE (curve b), CNT/CPE (curve d), modified CPE at DHB (curve e), and DHB/CNT/CPE (curve f). As can be seen, the anodic peak potential for CAP oxidation of CAP in surface of the unmodified CPE (curve b) is 430 mV, CNT/CPE (curve d) about 380 mV, and oxidation of CAP in surface of modified CPE with DHB (curve e) and DHB/CNT/CPE is about 250 mV. Potential of oxidation of CAP at modified electrodes with modifier is 180 mv more negative than unmodified electrodes with modifier. Similarly, when we compared the oxidation of CAP at the DHB/CPE (curve e) and DHB/CNT/CPE (curve f), there is a dramatic enhancement of the anodic peak current at DHB/CNT/CPE relative to the value obtained at the DHB/CPE. Also, modification with modifier decrease at the peak potential of oxidation of CAP compared with electrode without modifier. In other words, the data obtained clearly show that the combination of CNTs and mediator (DHB) definitely improve the characteristics of CAP oxidation. The DHB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0), without CAP in solution, exhibits well-behaved redox reactions (curve c) upon the addition of 0.5 mM CAP, and there is a dramatic enhancement of the anodic peak current and delete of back current (curve f), which can be related to the strong electrocatalytic effect of the DHB/CNT/CPE toward this compound. According to whatever displayed, the best electrocatalytic effect for CAP oxidation is showed at DHB/CNT/CPE (curve f).

Figure 3I shows that the process enhanced the peaks current with increase scan rate by linear sweep voltammetry, which the oxidation peak potential relocated toward more positive potentials with increase scan rate, according to kinetic limitation in the electrochemical reaction. As seen in Fig. 3I, there is a linear correlation between plots of peak height ( $I_p$ ) and the square root of scan ( $v^{1/2}$ ) in the range of 5–90 mV s<sup>-1</sup>, that at enough over potential, the process was diffusion rather than surface controlled. Sweep rate (Fig. 3I) exhibits the characteristic shape typical of an EC' process [53].

The inset of Fig. 3II shows a Tafel plot that was drawn from points shown in the linear sweep voltammetry. This part of the voltammogram, known as Tafel region, is affected by electron transfer kinetics between substrate (CAP) and surface-confined DHB, assuming the deprotonation of substrate as a sufficiently fast step. In this condition, the number of electron involved in the rate-determining step can be estimated from the slope of Tafel plot. A slope 0.181 V is obtained, indicating a one-electron transfer to be the rate-limiting step assuming a charge transfer coefficient of  $\alpha=0.67$ .

**Fig. 4** Chronoamperograms obtained at DHB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) for different concentrations of CAP. The numbers 1–6 correspond to 0, 0.4, 0.6, 1, 1.2, and 1.5 mM of CAP. Inset: *A* plots of  $I$  versus  $t^{-1/2}$  obtained from chronoamperograms, *B* plot of the slope of the straight lines against the CAP concentration. *C* Dependence of  $I_{\text{cat}}/I_1$  on  $t^{1/2}$  derived from the data of chronoamperograms



### Chronoamperometric investigation

The chronoamperometry as well as the other electrochemical methods was employed for the investigation of electrode processes at chemically modified electrodes. Chronoamperometric measurements of CAP at DHB/CNT/CPE were carried out by setting the working electrode potential at 350 mV for various concentrations of CAP (Fig. 4). For an electroactive material (CAP in this case) with a diffusion coefficient of  $D/\text{cm}^2 \text{ s}^{-1}$ , the current observed for the electrochemical

reaction at a mass transport limited condition is described by the Cottrell equation [53].

$$I = nFAC_b(D/\pi t)^{1/2} \quad (3)$$

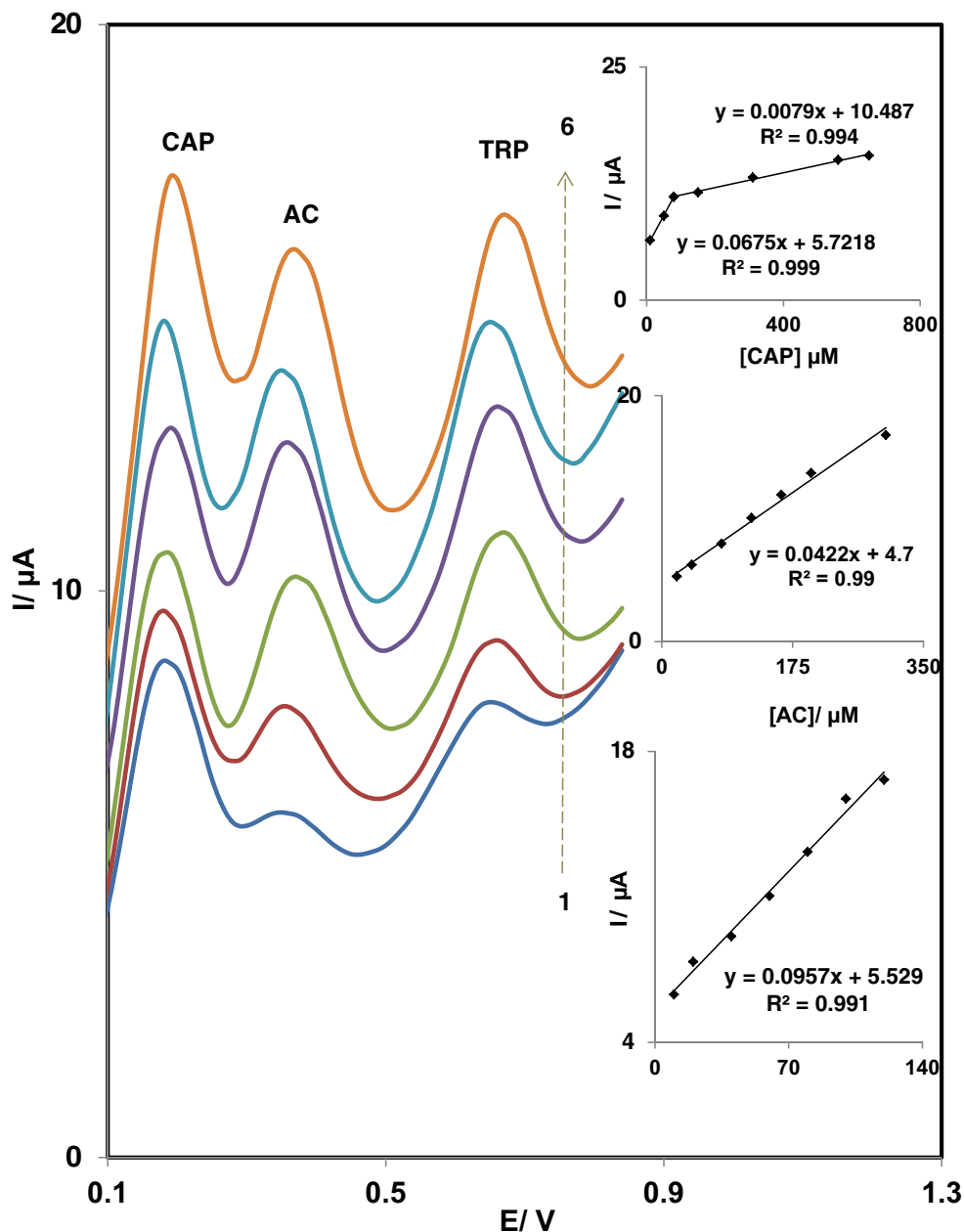
We have employed a plot of  $I$  versus  $t^{-1/2}$  that will be linear, then from the slopes plotted versus the CAP concentration, the value  $3.61 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  for  $D$  be obtained (Fig. 4b). Chronoamperometry can also be used to gain the catalytic

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

| Substrate | Modifier  | pH  | Scan rate (mV/s) | Limit of detection (M) | Dynamic range(M)                          | Ref.      |
|-----------|---|-----|------------------|------------------------|---|-----------|
| CNTPE     | Ferrocene-based polyamide   | 7.0 | 30               | $0.8 \times 10^{-7}$   | $0.2 \times 10^{-6} - 2 \times 10^{-4}$   | [43]      |
| CNTPE     | Ferrocenemonocarboxylic acid  | 7.0 | 10               | $0.3 \times 10^{-6}$   | $0.8 \times 10^{-6} - 65 \times 10^{-6}$  | [57]      |
| CNTPE     | <i>N</i> -(3,4-dihydroxyphenethyl)-3,5-dinitrobenzamide                 | 8.0 | 20               | $3.4 \times 10^{-8}$   | $6.4 \times 10^{-8} - 3.2 \times 10^{-4}$ | [58]      |
| CNTPE     | ( <i>E</i> )-3-((2-(2,4-dinitrophenyl)hydrazono)methyl)benzene-1,2-diol | 7.0 | 20               | $0.7 \times 10^{-7}$   | $0.2 \times 10^{-7} - 8.0 \times 10^{-4}$ | [60]      |
| CPE       | $\text{SiO}_2/\text{Al}_2\text{O}_3$                                    | 6.0 | 10               | $0.9 \times 10^{-7}$   | $3.0 \times 10^{-7} - 3.0 \times 10^{-2}$ | [59]      |
| GCE       | Hexacyanoferrate(II)  | 2.0 | –                | $0.2 \times 10^{-6}$   | $0.5 \times 10^{-6} - 6.0 \times 10^{-2}$ | [61]      |
| CPE       | NiO nanoparticle  | 7.0 | 10               | $0.7 \times 10^{-8}$   | $3.5 \times 10^{-8} - 5.5 \times 10^{-4}$ | [62]      |
| CPE       | Ferrocene carboxylic acid   | 7.0 | 10               | $0.96 \times 10^{-8}$  | $3 \times 10^{-8} - 24 \times 10^{-4}$    | [63]      |
| CNTPE     | <i>p</i> -Aminophenol   | 6.0 | 20               | $2 \times 10^{-8}$     | $5 \times 10^{-8} - 5 \times 10^{-5}$     | [64]      |
| CNTPE     | DHB   | 7.0 | 40               | $2.46 \times 10^{-6}$  | $7.0 \times 10^{-6} - 2.5 \times 10^{-3}$ | This work |

Carbon nanotube paste electrode (CNTPE)

**Fig. 5** DPVs of DHB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of CAP+AC+Trp in  $\mu\text{M}$ . The numbers 1–6 correspond to 50+20+10, 100+400+20, 200+80+40, 300+120+60, 400+160+80, 500+200+100, and 600+250+120, respectively



rate constant,  $k_h$ , for the reaction between CAP and the DHB/CNT/CPE, according to the method of Galus [56].

$$I_{cat}/I_1 = \pi^{1/2}(kct)^{1/2} \quad (4)$$

Where  $t$  is the time elapsed and  $c$  is the bulk concentration of CAP.

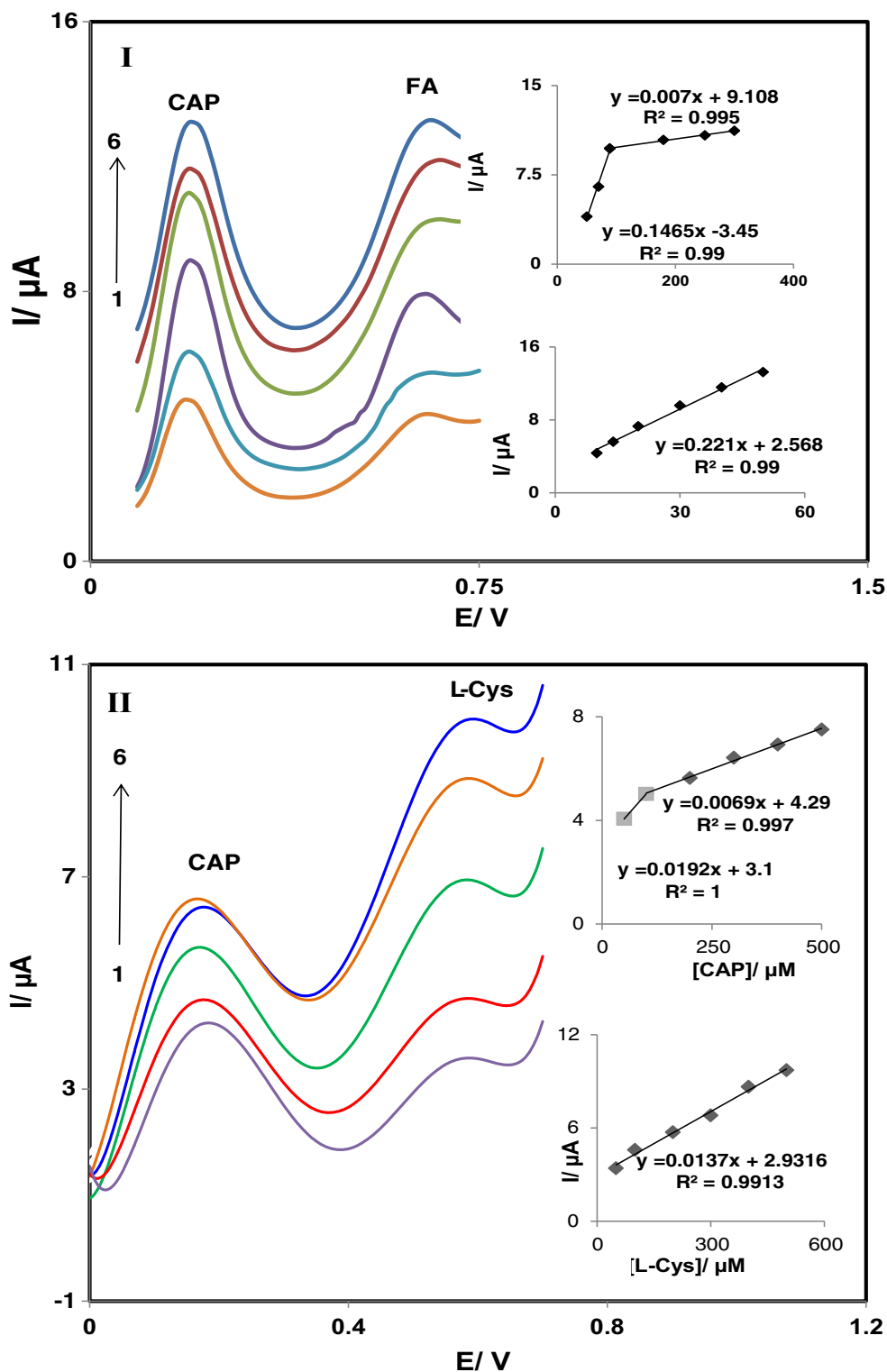
The average value of  $k$  was found at  $3.55 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ , using the value of the slopes, that is shown in Fig. 4c and mentioned equation. The value of  $k$  explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of CAP at the surface of DHB/CNT/CPE. Finally,

the heterogeneous rate constant ( $k'$ ) of catalytic reaction was calculated as  $k' = 2.49 \times 10^{-3} \text{ cm s}^{-1}$  (with  $\Gamma = 7.0 \times 10^{-8} \text{ mol cm}^{-2}$ ).

#### Calibration plot and limit of detection

Differential pulse voltammetry (DPV) can analyze the electrode reaction exactly and the resolution better than cyclic voltammetry. So the electrocatalytic peak current of CAP oxidation at the surface of DHB/CNT/CPE can be used for determining CAP in phosphate buffer solution containing various concentrations of CAP. DPVs can be used in estimating the limits of detection to determine trace amounts of CAP.

**Fig. 6** *I* DPVs of DHB/CNT/CPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of CAP+FA in  $\mu\text{M}$ . The numbers 1–6 correspond to 50+10, 70+14, 100+20, 150+30, 250+40, and 300+50, respectively; *II* DPVs of CAP+L-Cys in  $\mu\text{M}$ . The numbers 1–6 correspond to 50+50, 100+100, 200+200, 300+300, 400+400, and 500+500, respectively



In addition, the charging current contribution to the background current, which is a limiting factor in the analytical determination, is negligible in DPV mode.

The plot of peak current versus CAP concentration consisted of two linear segments with slopes of 0.0592 and

$0.0070 \mu\text{A} \mu\text{M}^{-1}$  in the concentration ranges of 7.0–100.0 and 100.0–2,500.0, respectively. The decrease in sensitivity (slope) in the second linear range is likely to be due to kinetic limitation [53]. The detection limit ( $3\sigma$ ) for CAP in the lower range region was found to be  $2.43 \mu\text{M}$ .



**Table 2** Determination of CAP, AC, Trp, FA, and L-Cys in real samples

| Sample | 1         |        |              | 2         |        |              | 3         |        |              |
|--------|-----------|--------|--------------|-----------|--------|--------------|-----------|--------|--------------|
|        | Substrate | Found  | Recovery (%) | Substrate | Found  | Recovery (%) | Substrate | Found  | Recovery (%) |
| CAP    | 600       | 601.24 | 100.21       | 500       | 499.06 | 99.81        | 400       | 398.00 | 99.5         |
| AC     | 250       | 249.85 | 99.94        | 200       | 200.98 | 100.49       | 160       | 160.8  | 100.5        |
| Trp    | 120       | 120.5  | 100.42       | 100       | 101.34 | 101.34       | 80        | 82.02  | 100.02       |
| CAP    | 200       | 198.47 | 99.23        | 150       | 150.45 | 100.33       | 100       | 98.97  | 98.97        |
| FA     | 40        | 41.03  | 102.57       | 30        | 29.51  | 98.36        | 20        | 20.59  | 102.95       |
| CAP    | 450       | 451.8  | 100.4        | 300       | 301.6  | 100.53       | 200       | 199.19 | 99.59        |
| L-Cys  | 450       | 448.9  | 99.75        | 300       | 298.10 | 99.37        | 200       | 200.85 | 104.2        |

Simultaneous determinations of CAP and some biological molecules

The ability of resolution of the electrochemical responses of different analyst is one of the important parameters at selecting a type of modified electrode. To the best of our knowledge, there is no prior report on the simultaneous determination of CAP, AC, TRP, FA, and L-Cys. Therefore, the main object of this work was to detect IP and some biological molecules simultaneously using DHB/CNT/CPE. This was performed by simultaneously changing the concentration of analytes, and recording the DPVs. Detection limit and linear range are comparable with values reported by other research groups for electrocatalytic oxidation of CAP at the surface of chemically modified electrodes by other mediators in Table 1 [57–61].

#### Simultaneous determination of CAP, AC, and Trp

DPVs were recorded simultaneously changing the concentration of captopril, acetaminophen, and tryptophan, which shows the oxidation potential of these three analytes at 0.18, 0.36, and 0.66 V, respectively (Fig. 5). They are exactly separable from each other. The detection limits of AC and Trp were obtained at 3.41 and 1.5  $\mu\text{M}$ , respectively, since the obtained value of slope for CAP is  $0.079 \mu\text{A } \mu\text{M}^{-1}$ , and alone, CAP is  $0.070 \mu\text{A } \mu\text{M}^{-1}$ ; these two values of slopes are very close to each other, so this electrode can easily be used for the determination of CAP in mixture of different concentration of AC and Trp simultaneously without significant interference. If the CAP signal is affected by the AC or Trp, the abovementioned slopes would be different.

#### Simultaneous determination of CAP and FA

Figure 6I shows responses of DPVs for mixture solution of different concentrations of captopril and folic acid simultaneously without interference. Oxidation potential of CAP and FA were recorded at 0.18 and 0.64  $\mu\text{M}$ , respectively. The limit

detection of FA obtained at 6.54 and the value of slope for CAP in this mixture and alone CAP is the same ( $0.0070 \mu\text{A } \mu\text{M}^{-1}$ ), so the modified electrode can be used for determination of CAP in prines of FA.

#### Simultaneous determination of CAP and L-cysteine

Since the voltammetric signals of CAP and L-Cys are close to each other at the bare electrode, we use DHB/CNT/CPE for the simultaneous determination of CAP and L-Cys by simultaneously changing their concentrations in the solution. DPV results show two well-distinguished anodic peaks at potentials of 0.17 and 0.56 V, indicating that the simultaneous determination of CAP and L-Cys is possible at the DHB/CNT/CPE (Fig. 6II). The limit detection of L-Cys obtained at 10.28  $\mu\text{M}$  and the value of slope for CAP in this mixture ( $0.0069 \mu\text{A } \mu\text{M}^{-1}$ ) and alone CAP ( $0.0070 \mu\text{A } \mu\text{M}^{-1}$ ) is very close together, so the modified electrode can be used for determination of CAP in prines of L-Cys without significant interference.

#### Interference study

The influence of various foreign species on the determination of CAP was investigated. The tolerance limit was taken as the maximum concentration. Of the foreign substances which caused an approximately 5 % relative error in the determination, acetaminophen, ascorbic acid, vitamin B<sub>6</sub>, uric acid, glycine, glucose, penicillin sodium, *N*-acetyl at 15 times concentration of CAP and epinephrine, dopamine, folic acid, tyrosine, and levodopa at 5 time concentration toward CAP did not show interference in the determination of CAP.

#### Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of CAP, AC, TRP, FA, and L-Cys in human blood serum samples. The sample was found to be free from

these analytes. Therefore, different amounts of analytes were spiked to the sample and analyzed by the proposed method. The results for determination of these analytes species in real samples are given in Table 2. Satisfactory recovery of the experimental results was found for these analytes.

#### The repeatability and stability of DHB/CNT/CPE

The electrode capability for the generation of a reproducible surface was examined by CVs data obtained in optimum solution pH from five separate samples that prepared DHB/CNT/CPE. The calculated RSD for various parameters was accepted as the criteria for a satisfactory surface reproducibility (1–4 %). This degree of reproducibility is virtually the same as that expected for the renewal or ordinary carbon paste surface. In addition, the long-term stability of the DHB/CNT/CPE was tested over a 3-week period. When CVs were recorded after the modified electrode was stored in an atmosphere at room temperature, the peak potential for CAP oxidation was unchanged and the current signals showed only less than 2.1 % decrease of the initial response. The antifouling properties of modified electrode towards CAP oxidation and its oxidation products were investigated by recording the cyclic voltammograms of modified electrode before and after using in the presence of CAP. Cyclic voltammograms were recorded in the presence of CAP after having cycled the potential for 20 cycles at a scan rate  $100 \text{ mV s}^{-1}$ . The peak potentials were unchanged to positive values and the currents decreased by less than 2.1 %. Therefore, at the surface of DHB/CNT/CPE, not only the sensitivity increase, but the fouling effect of the analyte and its oxidation product also decreases. However, we regenerated the surface of DHB/CNT/CPE before each experiment.

#### Conclusions

A stable modified carbon nanotube paste electrode (modification with CNTs and DHB) with long stability and excellent reproducibility was fabricated. The modified electrode has been shown to be promising for determination of CAP with many desirable properties including high stability, selectivity, good reproducibility and repeatability, fast response time, together with the ease of preparation, low cost, and surface regeneration, making the proposed modified electrode very useful for accurate determination of CAP, AC, Trp, FA, and L-Cys in real samples. Thus, the electrode could electrochemically discriminate the sensing of CAP, AC, TRP, FA, and L-Cys. High sensitivity and selectivity of the voltammetric responses, and low detection limit, together with the ease of preparation and surface regeneration, make the proposed

modified electrode very useful for accurate determination of these substrates in human serum samples.

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