

Full Paper

Selective Voltammetric Determination of Carbidopa in the Presence of Uric Acid Using a Modified Carbon Nanotube Paste Electrode

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Abstract- This paper reports the selective determination of carbidopa (CD) in the presence of uric acid (UA) using 2,7-bis(ferrocenyl ethyl)fluoren-9-one modified carbon nanotube paste electrode (2,7-BFCNPE) in 0.1 M phosphate buffer solution (PBS) pH 7.0. In PBS of pH 7.0, the oxidation current increased linearly with two concentration intervals of CD, one is 0.2 to 22.0 μM and, the other is 22.0 to 750.0 μM . The detection limit (3σ) obtained by DPV was 68.0 nM. The practical application of the modified electrode was demonstrated by determining CD in urine samples .

Keywords- Carbidopa, Uric Acid, Carbon Nanotube, Electrocatalysis, Modified Electrode

1. INTRODUCTION

Carbon nanotubes (CNTs) are considered as a novel form of carbon materials in the last two past decades [1-4]. Recently, carbon nanotubes have also been incorporated into the electrochemical sensors[5-8]. While they have many of the same properties as other types of carbon, they offer unique advantages including enhanced electronic properties, a large edge plane/basal plane ratio, and rapid kinetics of the electrode processes. Therefore, in comparison to the traditional carbon electrodes, CNT-based sensors generally have higher sensitivities, lower limits of detection, and faster electron transfer kinetics [9-13].

Drug analysis is one of the important tools for drug quality control. Therefore, the development of simple, sensitive, rapid and reliable method for the determination of drug is of great importance [14-20].

Parkinson's disease is believed to be related to low levels of the neurotransmitter dopamine in the brain. Therefore, the dopamine precursor levodopa (LD) [3-(3,4-dihydroxyphenyl)-L-alanine] is employed for its treatment. In order to enhance its pharmacological effect, levodopa is generally associated with a peripheral aromatic decarboxylase inhibitor such as carbidopa (CD) [(S)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid]. This combination is also used to treat tumors, spasms and poor muscle control caused by CO and manganese intoxication, as well as in ophthalmology (amblyopia and strabismus) [21-23]. Accordingly, the development of an analytical method is very important to control the content of these catecholamines in pharmaceuticals. Different techniques have been employed for the determination of CD in pharmaceutical formulations. Voltammetry is considered as an important electrochemical technique utilized in electroanalytical chemistry because, it provides low cost, sensitivity, precision, accuracy, simplicity and rapidity [24, 25].

Uric acid (UA) is the main end product of purine metabolism in the human body. Increased consumption of serum, UA acts as a scavenger of radicals and thus preventing from Parkinson's disease [26]. Low concentration of UA associated with multiple sclerosis [27]. Thus, accurate determination of UA is essential in the clinical point of view. Several methods for the determination of UA have been reported. One of the most reliable ones is those based on electrochemical methods [28-35].

With respect to the electrochemical behaviors, CD and UA always considered as interferences to each other for determination of any one, because voltammetric response of the electrode to CD is commonly very close to that of UA, and their signals usually overlap. Therefore, the development of a rapid, selective and simple method is very important for their determinations in routine analysis.

Therefore, in the present work, we describe the preparation of a new electrode composed of CNPE modified with 2,7-bis(ferrocenyl ethyl)fluoren-9-one (2,7-BFCNPE) and investigate its performance for the electrocatalytic determination of CD in aqueous solutions. We also

evaluate the analytical performance of the modified electrode for quantification of CD in the presence of UA .

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, The Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the 2,7-BFCNPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 691 pH/Ion meter was used for pH measurements .

All solutions were freshly prepared with double distilled water. CD, UA and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Graphite powder and paraffin oil (DC 350, density = 0.88 g cm^{-3}) as the binding agent (both from Merck) were used for preparing the pastes. 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 10 to 30 μm were prepared from Nanostructured & Amorphous Materials, Inc. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-11.0. 2,7-BF was synthesized in our laboratory as reported previously [36].

2.2. Preparation of the electrode

The 2,7-BFCNPEs were prepared by hand mixing 0.01 g of 2,7-BF with 0.89 g graphite powder and 0.1 g CNTs 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. 3.4 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, 2,7-BF modified CPE electrode (2,7-BFCPE) without CNTs, CNT paste electrode (CNPE) without 2,7-BF, and unmodified CPE in the absence of both 2,7-BF and CNT were also prepared in the same way .

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of 2,7-BFCNPE

We have previously shown that a carbon paste electrode spiked with 2,7-BF is constructed by the incorporation of 2,7-BF in a graphite powder paraffin oil matrix. The

experimental results show that well-defined and reproducible anodic and cathodic peaks related to 2,7-bis(ferrocenyl ethyl)fluoren-9-one/2,7-bis(ferricenium ethyl)fluoren-9-one (Fc/Fc⁺) redox system, which show a quasi-reversible behavior in an aqueous medium [37]. The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH 7.0 from five separately prepared 2,7-BFCNPEs (Table 1). The calculated RSD for various parameters accepted as the criteria for a satisfactory surface reproducibility (about 1–4%), which is virtually the same as that expected for the renewal or ordinary carbon paste surface [36]. However we regenerated the surface of 2,7-BFCNPE before each experiment according to our previous result [36].

Table 1. Cyclic voltammetric data obtained for constructed 2,7-BFCNPE in 0.1 M PBS (pH 7.0) at 10 m Vs⁻¹

E_{pa} (V)[a]	E_{pc} (V)	$E_{1/2}$ (V)	ΔE_p (V)	I_{pa} (μ A)	I_{pc} (μ A)
0.320[b]	0.255	0.287	0.065	0.79	0.77

[a] vs. Ag/AgCl/KCl (3.0 M) as reference electrode

[b] All the ‘ \pm ’ values are RSD% (n = 5)

3.2. Electrocatalytic oxidation of CD at a 2,7-BFCNPE

Fig. 1 depicts the CV responses for the electrochemical oxidation of 0.15 mM CD at unmodified CPE (curve b), CNPE (curve d), 2,7-BFCPE (curve e) and 2,7-BFCNPE (curve f). As it is seen, while the anodic peak potential for CD oxidation at the CNPE, and unmodified CPE are 700 and 750 mV, respectively, the corresponding potential at 2,7-BFCNPE and 2,7-BFCPE is ~320 mV. These results indicate that the peak potential for CD oxidation at the 2,7-BFCNPE and 2,7-BFCPE electrodes shift by ~380 and 430 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 2,7-BFCNPE shows much higher anodic peak current for the oxidation of CD compared to 2,7-BFCPE, indicating that the combination of CNTs and the mediator (2,7-BF) has significantly improved the performance of the electrode toward CD oxidation. In fact, 2,7-BFCNPE in the absence of CD exhibited a well-behaved redox reaction (Fig. 1, 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 0.15 mM CD (curve f), which can be related to the strong electrocatalytic effect of the 2,7-BFCNPE towards this compound [37].

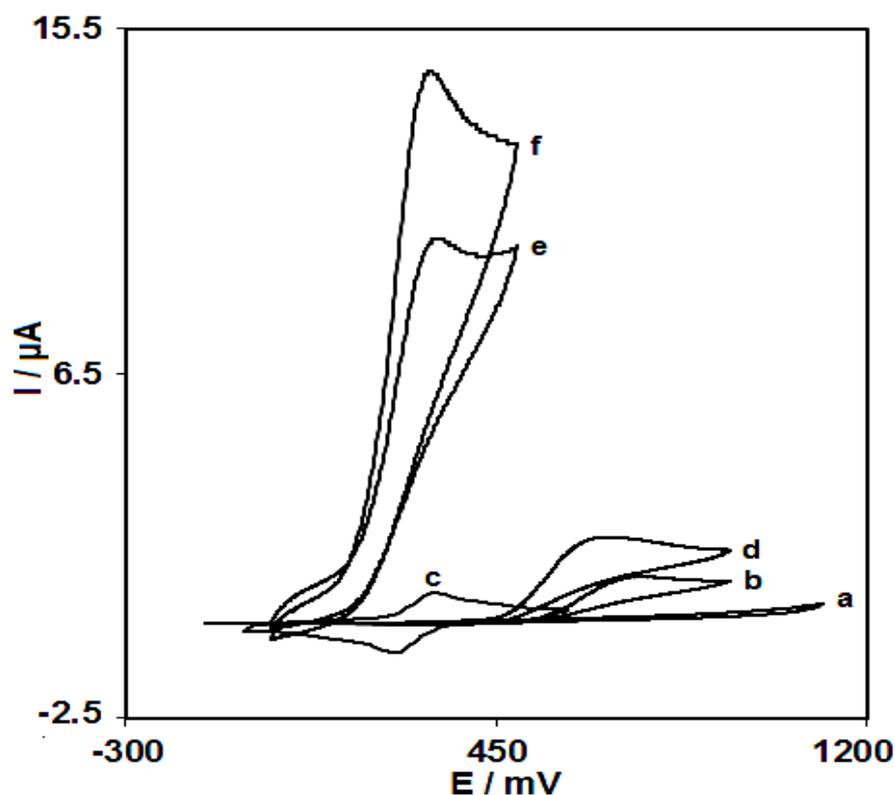


Fig. 1. CVs of (a) unmodified CPE in 0.1 M PBS (pH 7.0) at scan rate of 10 mV s^{-1} ; (b) as (a) +150.0 μM CD; (c) as (a) at the surface of 2,7-BFCNPE; (d) as (b) at the surface of CNPE; (e) as (b) at the surface of 2,7-BFCPE; (f) as (b) at the surface of 2,7-BFCNPE

The effect of scan rate of potential on the electrocatalytic oxidation of CD at the 2,7-BFCNPE was investigated by cyclic voltammetry. A plot of peak height (I_p) vs. the square root of scan rate ($v^{1/2}$) was found to be linear in the range of $10\text{--}60 \text{ mV s}^{-1}$ (Fig. 2A), suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled [37]. A plot of the scan rate-normalized current ($I_p/v^{1/2}$) vs. scan rate (Fig. 2B) exhibits the characteristic shape typical of an EC' process [37].

Tafel plot was drawn from data from the rising part of the current–voltage curve, which were recorded at a scan rate of 10 mV s^{-1} (Fig. 3). A slope of 0.123 V was obtained, indicating that a one electron transfer was rate limiting, assuming a transfer coefficient of $\alpha = 0.52$.

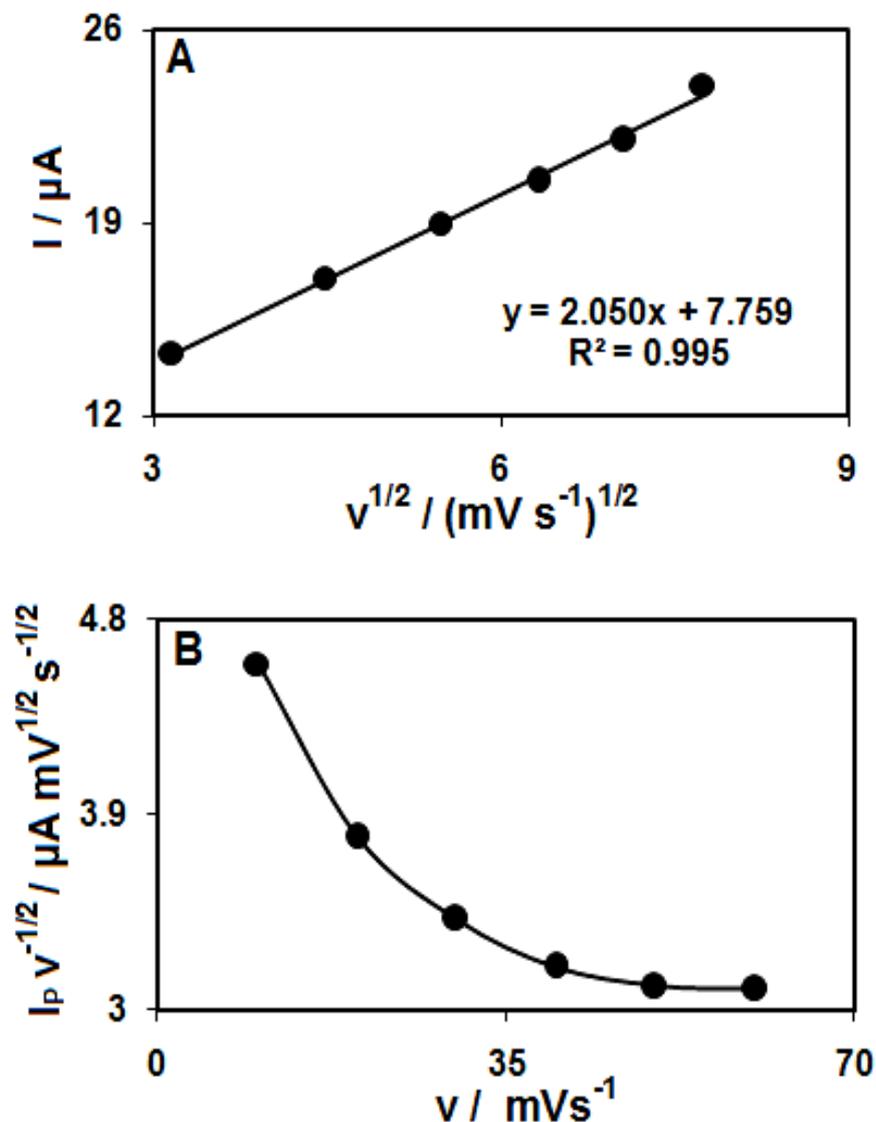


Fig. 2. (A) Variation of anodic peak current vs. $v^{1/2}$ obtained from linear sweep voltammograms of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing 150.0 μM CD at various scan rates (10, 20, 30, 40 50 and 60 mV s^{-1}); (B) Normalized current ($I_p/v^{1/2}$) vs. v .

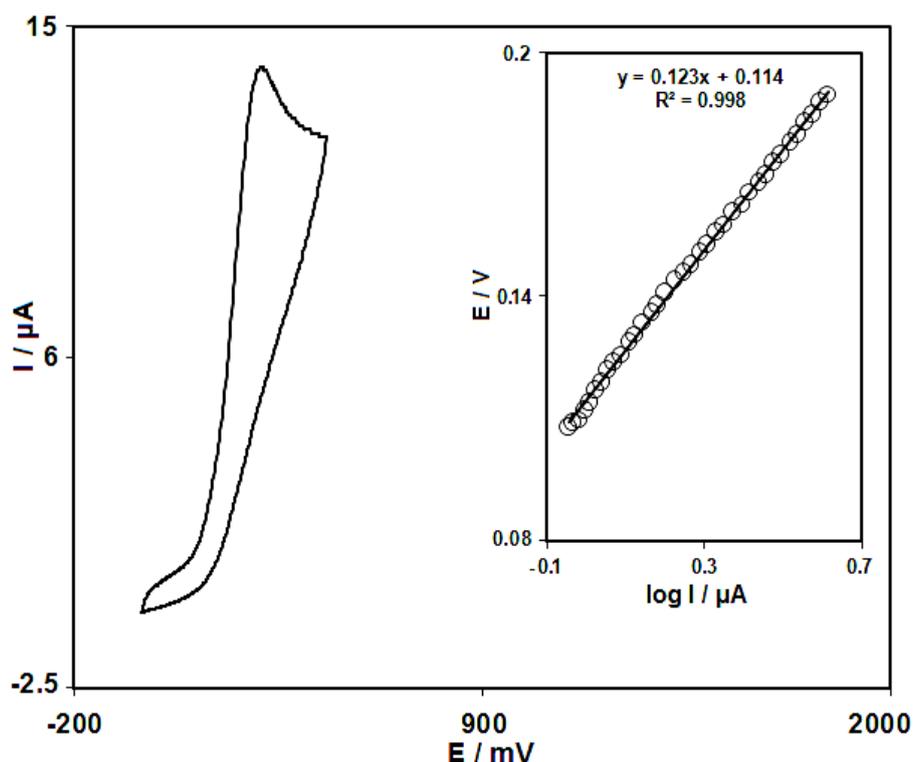


Fig. 3. Cyclic voltammogram (at 10 mV s^{-1}) of a 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing 0.15 mM CD. The inset shows the Tafel plot derived from the cyclic voltammogram

3.3. Chronoamperometric measurements

Chronoamperometric measurements of CD at 2,7-BFCNPE were carried out by setting the working electrode potential at 0.4 V vs. Ag/AgCl/KCl (3.0 M) for the various concentration of CD in buffered aqueous solutions (pH 7.0) (Fig. 4). For an electroactive material (CD 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 equation [37]. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of CD (Fig. 4A). The slopes of the resulting straight lines were then plotted vs. CD concentration (Fig. 4B). From the resulting slope and Cottrell equation, the mean value of the D was found to be $3.1 \times 10^{-6} \text{ cm}^2/\text{s}$.

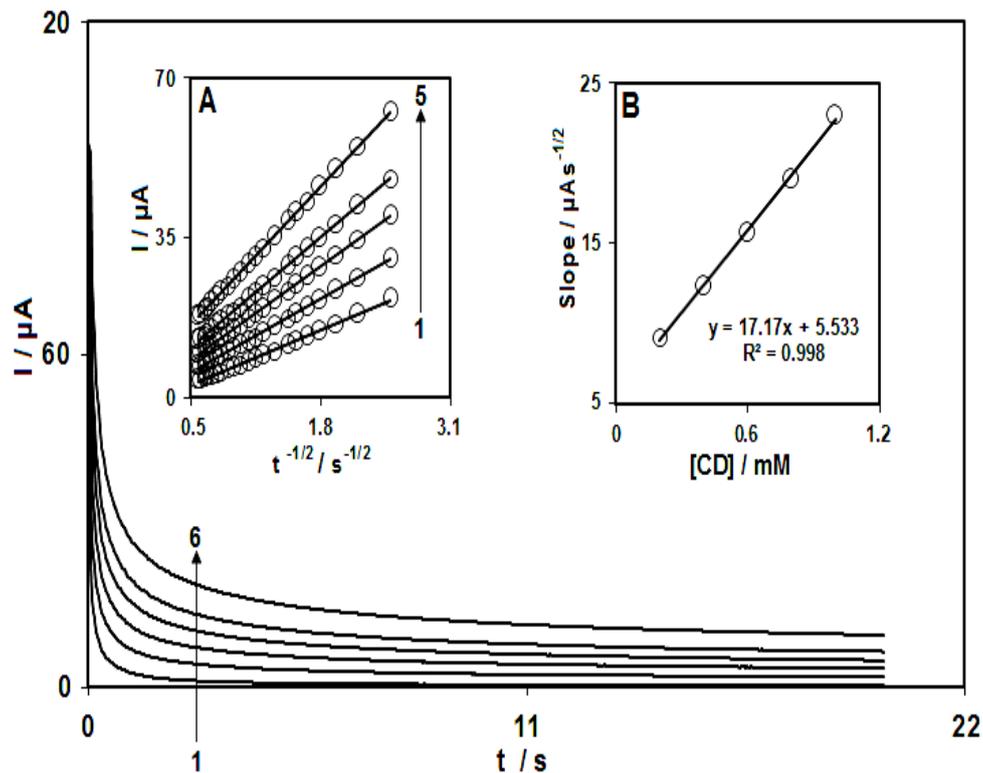


Fig. 4. Chronoamperograms obtained at 2,7-BFCNPE in 0.1 M PBS (pH 7.0) for different concentration of CD by setting potential step at 400 mV. The numbers 1–6 correspond to 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mM of CD. Insets: A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2–6 and B) Plot of the slope of the straight lines against the CD concentration

3.4. Calibration plot and limit of detection

DPV method was used to determine the concentration of CD. The plot of peak current vs. CD concentration consisted of two linear segments with slopes of 0.402 and 0.022 $\mu\text{A } \mu\text{M}^{-1}$ in the concentration ranges of 0.2 to 22.0 μM and 22.0 to 750.0 μM , respectively (Fig. 5). The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of CD was found to be 68.0 nM.

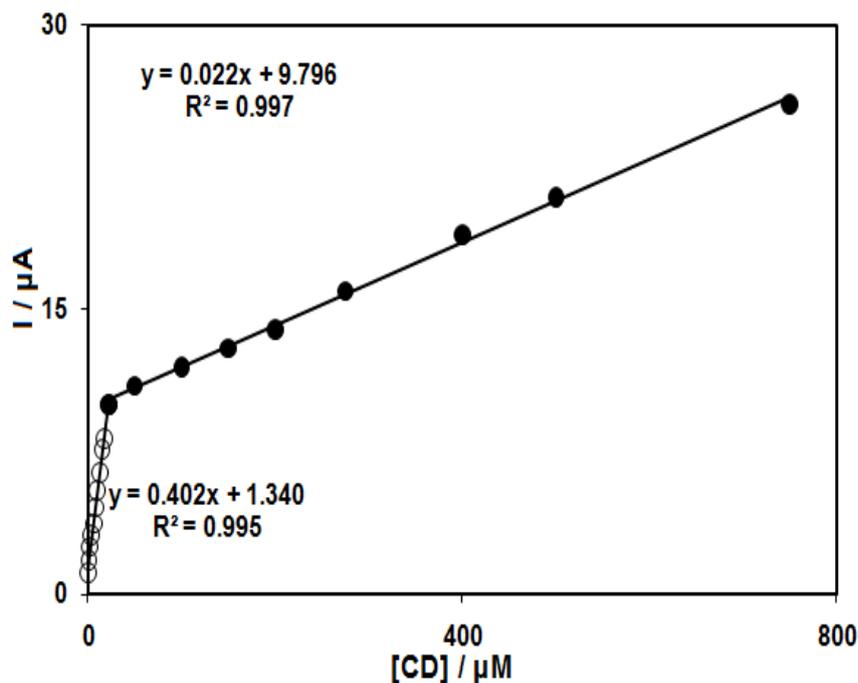


Fig. 5. The plots of the electrocatalytic peak current as a function of CD obtained from DPVs of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of CD (0.2, 1.0, 2.5, 4.0, 6.0, 8.0, 10.0, 12.0, 15.0, 17.5, 22.0, 50.0, 100.0, 150.0, 200.0, 275.0, 400.0, 500.0 and 750.0 μM)

3.5. Simultaneous determination of CD, and UA

To our knowledge, there is no report on the simultaneous determination of CD and UA using 2,7-BFCNPE. Therefore, the main object of this study was to detect CD and UA simultaneously using 2,7-BFCNPE. This was performed by simultaneously changing the concentrations of CD and UA, and recording the DPVs. The voltammetric results showed well-defined anodic peaks at potentials of 290 and 450 mV, corresponding to the oxidation of CD and UA respectively, indicating that simultaneous determination of these compounds is feasible at the 2,7-BFCNPE as shown in Fig. 6 .

The sensitivity of the modified electrode towards the oxidation of CD was found to be $0.39 \mu\text{A} \mu\text{M}^{-1}$. This is very close to the value obtained in the absence of ($0.42 \mu\text{A} \mu\text{M}^{-1}$, see Section 3.4), indicating that the oxidation processes of these compounds at the 2,7-BFCNPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences .

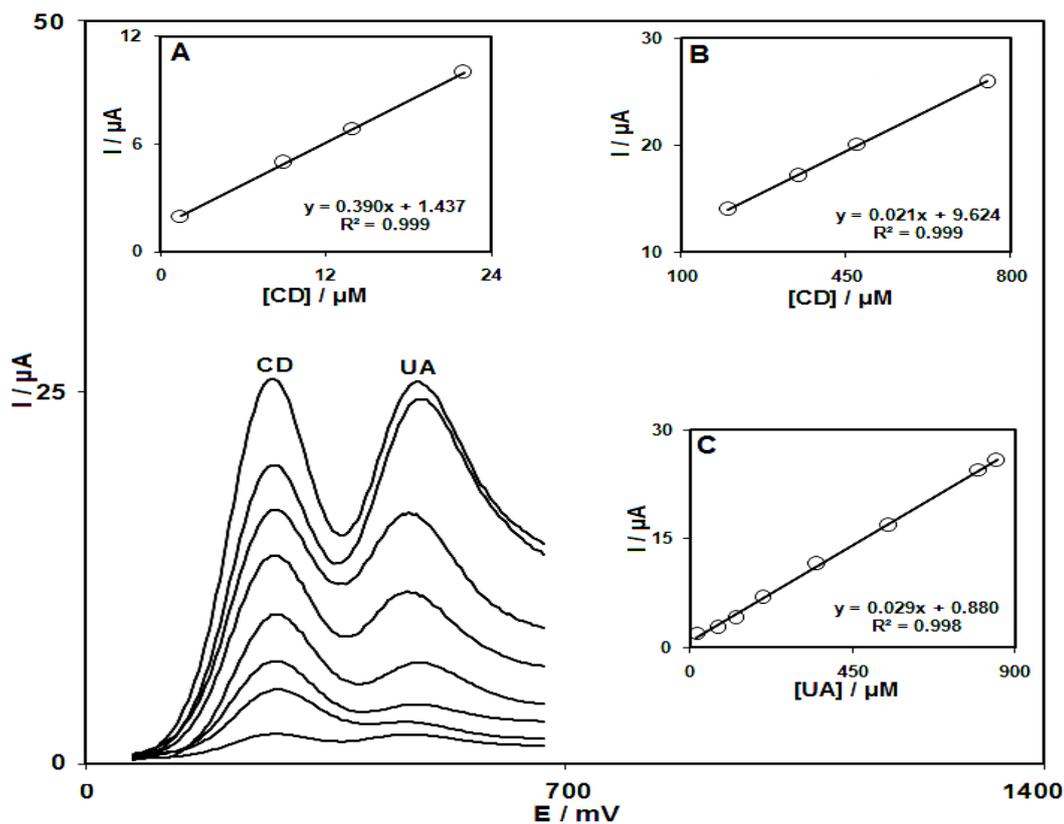


Fig. 6. DPVs of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of CD+UA in μM , from inner to outer: 1.5+17.0, 9.0+75.0, 14.0+125.0, 22.0+200.0, 200.0+350.0, 350.0+550.0, 475.0+800.0 and 750.0+850.0 respectively. (A), (B) and (C) are plots of I_p vs. CD and UA concentrations, respectively

3.6. Real sample analysis

3.6.1. Determination of CD and UA in urine samples

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of CD and UA in urine samples. The results are given in Table 2. Satisfactory recovery of the experimental results was found for CD and UA. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 2. The application of 2,7-BFCNPE for simultaneous determination of CD and UA in urine samples (n=5)

Sample	Spiked ($\mu\text{mol/L}$)		Found ($\mu\text{mol/L}$)		Recovery (%)		R.S.D. (%)	
	CD	UA	CD	UA	CD	UA	CD	UA
Urine	0	0	ND	23.0	-	-	-	2.4
	5.0	10.0	4.9	33.5	98.0	101.5	1.7	3.4
	10.0	12.5	10.3	35.1	103.0	98.9	2.3	2.8
	15.0	15.0	14.8	38.9	98.7	102.4	2.1	1.9
	20.0	17.5	20.2	39.9	101.0	98.5	3.1	2.7

4. CONCLUSIONS

In the present study, carbon-paste electrode modified with 2,7-BF and CNTs was used for the determination of CD in the presence of UA. The CV and DPV investigations showed effective electrocatalytic activity of the modified electrode in lowering the anodic over potential for the oxidation of CD and complete resolution of its anodic wave from UA. The detected potential differences of 160 mV between CD–UA, is large enough to allow simultaneous determination of CD and UA in mixtures without significant interferences.

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