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Electrocatalytic Oxidation of Levodopa at a Ferrocene Modified Carbon Nanotube Paste Electrode

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Levodopa (LD), the biological precursor of catecholamines, is the most widely prescribed drug in the treatment of Parkinson's disease. The present work presents a proposal for the application of a carbon paste electrode modified by ferrocene (FC) and carbon nanotubes as an electrochemical sensor for monitoring LD. Using the electrooxidation of LD at +0.370 in phosphate buffer pH 7.0 on a ferrocene modified carbon nanotubes paste electrode (FCMCNPE) it is possible to obtain a linear calibration curve from 2.0×10^{-6} to 5.0×10^{-4} M and a detection limit of 1.2 μM. The method was successfully applied to the determination of LD in urine sample without any pre-treatment.

Keywords: Levodopa, Ferrocene, Carbon nanotube, Electrocatalysis

1. INTRODUCTION

Carbon nanotubes can be used to promote electron transfer reactions when used as electrode material in electrochemical devices, electrocatalysis and electroanalysis processes due to their significant mechanical strength, high electrical conductivity, high surface area, good chemical stability, as well as relative chemical inertness in most electrolyte solutions and a wide operation potential window [1]. The electronics properties of these nanomaterials have been exploited as means of promoting the electron transfer reaction for a wide range of molecules and biological species including; insulin [2], carbohydrates [3], hydrogen peroxide [4], glucose [5], norepinephrine [6], aminophenol [7], morin [8], cytochrome C [9], promethazine [10], thiols [11], methyldopa [12], epinephrine [13] and nicotinamide adenine dinucleotide [14].

LD [3-(3,4-dihydroxyphenyl)-L-alanine], a precursor of the neurotransmitter dopamine, is the most widely prescribed drug in the treatment of Parkinson's disease. People with Parkinson's disease have depleted levels of dopamine, which causes tremor, muscle stiffness or rigidity, slowness of movement (bradykinesia) and loss of balance. Dopamine cannot be administered directly because it does not cross the blood brain barrier readily, while its precursor LD is given orally and is easily absorbed through the bowel and converted into dopamine by decarboxylase. Then, LD is used to increase dopamine in the brain, which reduces the symptoms of Parkinson's disease.

Nevertheless, elevated levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias [15]. Therefore, in order to achieve a better curative effect and a lower toxicity, it is very important to rapidly control the content of LD and its inhibitors and impurities in biological fluids and pharmaceutical formulations. Several techniques have been reported in the literature for the determination of LD such as spectrophotometry [16-18], spectrofluorimetry [19], ion-selective electrode [20], NMR spectroscopy [21],flow injection analysis (FIA) [22,23], high performance liquid chromatography (HPLC) [24, 25] and capillary electrophoresis[26, 27]. Nevertheless, each technique has often suffered from diverse disadvantages with regard to cost and selectivity, the use of organic solvents, complex sample preparation procedures or long analysis time. Electrochemical methods provide useful alternatives since they allow faster, cheaper and safer analysis

To our knowledge, no study has reported the electrocatalytic determination of LD by using carbon nanotube paste electrodes. Also, no paper has reported FC as catalyst for electrocatalysis of LD. Thus, in continuation of our studies concerning the preparation of chemically modified electrodes [28-32] in this paper, we described initially the preparation and suitability of a ferrocene modified carbon nanotube paste electrode (FCMCNPE) as a new electrocatalyst in the electrocatalysis and determination of LD in an aqueous buffer solution. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of LD in real samples, we examined this method for the voltammetric determination of LD in urine sample.

2. EXPERIMENTAL PART

2.1. Apparatus and reagents

All electrochemical measurements were performed using BHP 2063$^+$ Electrochemical Analysis System, Behpajooh, Iran potentiostat/galvanostat coupled whit a Pentium IV personal computer connected to a HP laser jet 6L printer. An Ag / AgCl / KCl 3 M, a platinum wire, and a FCMCNPE 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. LD was purchased from Merck. 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 Nanostruc-tured & Amorphous Materials (USA). The buffer solutions were prepared from ortho phosphoric acid and its salts in the pH range 2.0-11.0.

2.2. Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by dissolve 0.01 g of FC in diethyi ether and hand mixing with 89-times its weight of graphite powder and 10-times its weight of carbon nanotube with a mortar and pestle. The solvent was evaporated by stirring. A 70:30 (w/w) mixture of FC spiked carbon nanotube 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 (ca. 2 mm i.d. and 10 cm long). 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, prepared in the same way without adding FC and carbon nanotube to the mixture and was used for comparison purposes.

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behavior of FCMCNPE

Cyclic voltammetry was employed for investigation the electrochemical properties of FCMCNPE in a pure buffered aqueous solution (pH 7.0). The cyclic voltammogram (Fig. 1), exhibits an anodic and corresponding cathodic peaks with E_{pa}=0.370 Vand E_{pc}=0.265 V vs. Ag / AgCl / KCl 3 M. The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc^+ redox couple with quasireversible behavior, because of the peak separation potential, $\Delta E_p = (E_{pa} – E_{pc})$, is greater than that 59/n mV expected for a reversible system. Also, the obtained result from cyclic voltammetry of this modified electrode in various buffered solutions does not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of Fc/Fc^+ in FCMCNPE is independent on the pH of aqueous solution.

The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH from five separately prepared FCMCNPEs (Table 1). The calculated RSDs for various parameters 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 FCMCNPE was tested over a three-week period. The cyclic voltammetry of LD at the surface of FCMCNPE after the modified electrode was stored in an atmosphere at room temperature shows the oxidation peak potential of LD was unchanged and the anodic peak current was only decreased less than 2.4 % of the initial oxidation peak current. The antifouling properties of modified electrode toward LD and its oxidation product were investigated by recording the cyclic voltammograms of this modified electrode before and after using in the presence of LD.

The cyclic voltammetry of LD at the surface of FCMCNPE after 15 repetition cycles at a scan rate 20 mVs^{-1} shows the oxidation peak potential of LD was not changed and the anodic peak current was decreased by less than 3.3 %. However we regenerated the surface of FCMCNPE before each experiment.

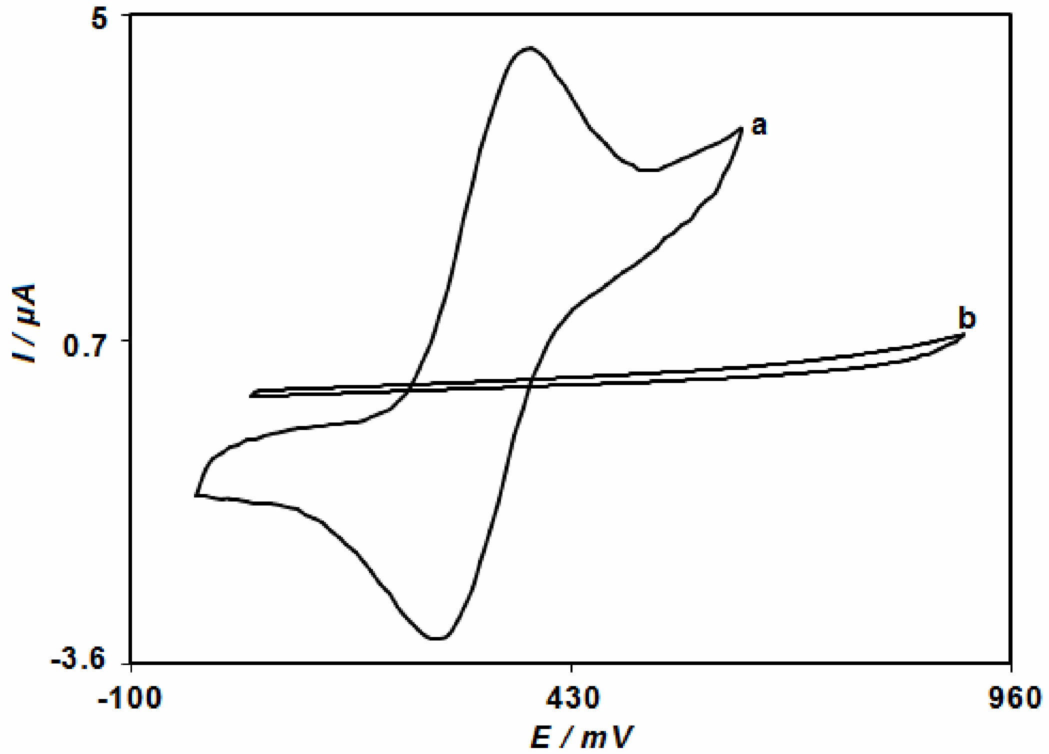

Figure 1. The cyclic voltammograms of a) FCMCNPE and b) bare CPE in 0.1 M phosphate buffer solution (pH 7.0) at a scan rate 20 mV s^{-1}.

Table 1. Cyclic voltammetric data obtained for constructed FCMCNPE in 0.1 phosphate buffer solution (pH 7.0) at 20 mVs^{-1}.

E_{pa} (V) [a]	E_{pc} (V) [a]	$E_{1/2}$ (V) [a]	ΔE_{pa} (V) [a]	I_{pa} (μA)	I_{pc} (μA)	Γ_a (mol cm^{-2})	Γ_c (mol cm^{-2})
0.370	0.265	0.3175	0.105	4.55	3.27	1.85×10^{-9}	1.45×10^{-9}
(0.75) [b]	(0.67) [b]	(0.78) [b]	(0.81) [b]	(2.45) [b]	(2.12) [b]	(3.4) [b]	(2.8) [b]

[a] Versus Ag / AgCl / KCl 3 M as reference electrode
[b] The values in paranthesis indicate the calculated RSD

3.2. Electrochemistry of LD at FCMCNPE

Fig. 2 depicts the cyclic voltammetric responses from the electrochemical oxidation of 200 μM LD at FCMCNPE (curve f), FC modified CPE (FCMCPE) (curve e), CNPE (curve d) and bare CPE (curve a). As can be seen, the anodic peak potential for the oxidation of LD at FCMCNPE (curve f) and FCMCPE (curve e) is about 375 mV, while at the CNPE (curve d) peak potential is about 580 mV, and at the bare CPE (curve b) peak potential is about 630 mV for LD. From these results it is concluded that the best electrocatalytic effect for LD oxidation is observed at FCMCNPE (curve f). For example, the results are shown that the peak potential of LD oxidation at FCMCNPE (curve f) shifted by about 315 and 365 mV toward the negative values compared with that at a CNPE (curve d) and bare CPE (curve b), respectively. Similarly, when we compared the oxidation of LD at the FCMCPE (curve e) and FCMCNPE (curve f), there is a dramatic enhancement of the anodic peak current at FCMCNPE relative to the value obtained at the FCMCPE. In the other words, the data obtained clearly show that the combination of carbon nanotube and mediator (FC) definitely improve the characteristics of LD oxidation. The FCMCNPE in 0.1M phosphate buffer (pH 7.0), without LD in solution, exhibits a well-behaved redox reaction (curve c) upon the addition of 200 μM LD, the anodic peak current of mediator was greatly increased, while the corresponding cathodic peak was disappear on the reverse scan of the potential (curve f). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes [33].

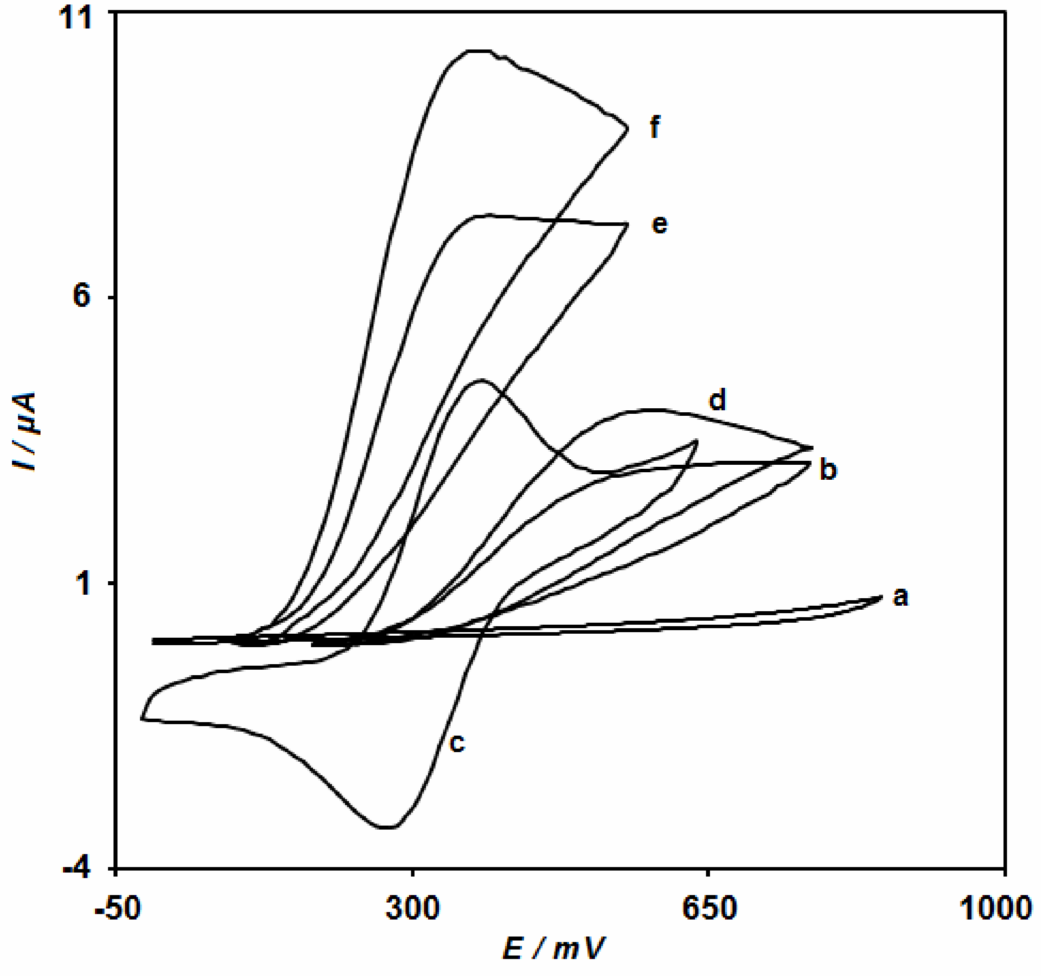

Figure 2. Cyclic voltammograms of (a) CPE in 0.1M PBS (pH 7.0) at scan rate 20 mV s^{-1} and (b) as (a) + 200 μM LD; (c) as (a) and (d) as (b) at the surface of FCMCNPE and CNPE respectively. Also, (e) and (f) as (b) at the surface of FCMCPE and FCMCNPE respectively.

The effect of scan rate on the electrocatalytic oxidation of 200 μM LD at the FCMCNPE was investigated by cyclic voltammetry. The oxidation peak potential shifts with increasing scan rates towards a more positive potential, confirming the kinetic limitation of the electrochemical reaction. Also, a plot of peak height (Ip) against square root of scan rate ($v^{1/2}$), in range of 10-60 mVs^{-1}, was constructed (Fig. 3A, curve a), which was found to be linear, suggesting that at sufficient overpotentials the process is diffusion rather than surface controlled. A plot of the sweep rate normalized current (Ip/v $^{1/2}$) versus sweep rate (Fig. 3A, curve b) exhibits the characteristic shape typical of an EC_{cat} process.

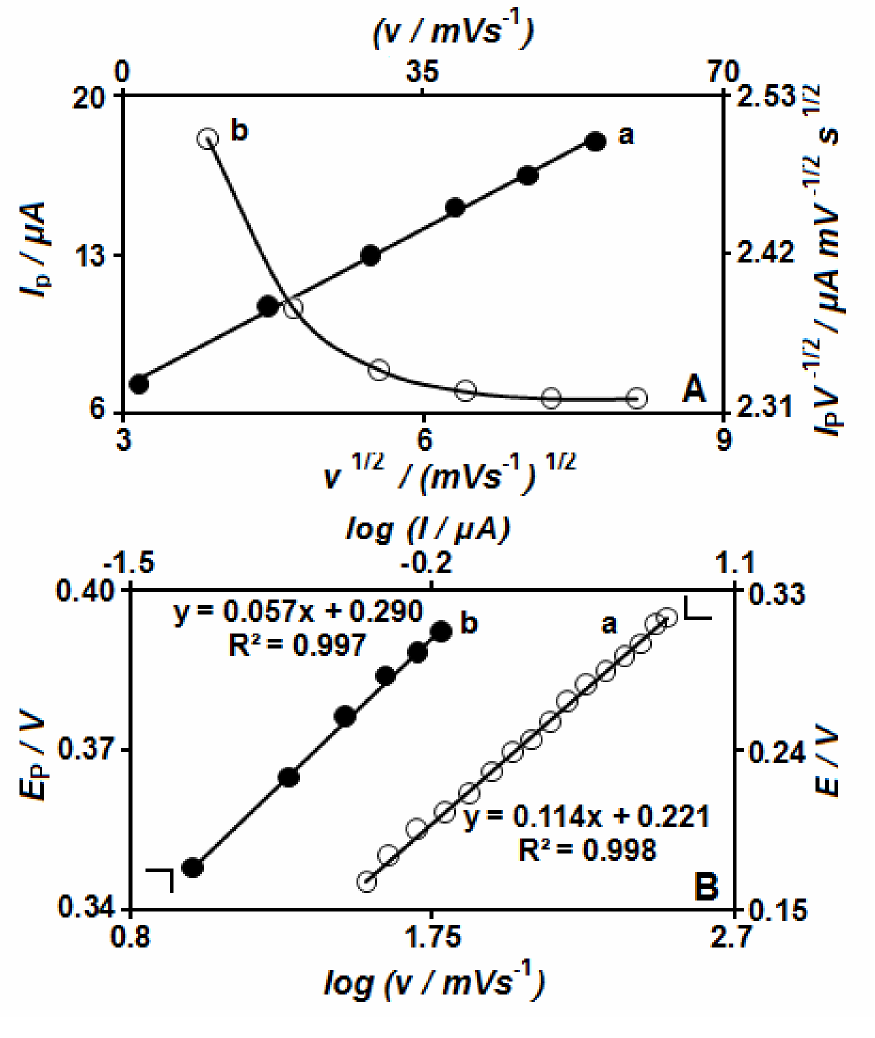

Figure 3. (A) Curve a, variation of the electrocatalytic current (Ip) with the square root of scan rate and curve b, variation of the scan rate- normalized current (Ip/*v* $^{1/2}$) with scan rate. (B) Curve a, Tafel plot derived from the rising part of voltammogram recrded at a scan rate 20 mVs^{-1} and curve b, plot of E_p vs. log *v*.

From the slope of E_p vs. log ν as is shown in Fig. 3B, curve b, the Tafel slope can also be obtained from the following equation [34]:

$$E_p = (b/2) \log \nu + \text{constant} \qquad (1)$$

The slope of E_p vs. log ν plot is b/2, where b indicates the Tafel slope. The slope of E_p vs. log ν plot is $\partial E_p / \partial (\log \nu)$ and was found to be 0.057 V in this work, so, b = 2 ×0.057 = 0.114 V. The value of Tafel slope indicates that a one-electron transfer process is the rate limiting step assuming a transfer coefficient of α about 0.48.

Fig. 3B, curve a, shows a Tafel plot that was drawn from data of the rising part of the current–voltage curve recorded at a scan rate of 20 mVs^{-1}. This part of voltammogram, known as Tafel region, is affected by electron transfer kinetics between LD and FC, 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 0.114 $Vdecade^{-1}$ is obtained indicating a one electron transfer to be rate limiting assuming a transfer coefficient of α = 0.48.

3.3. Chronoamperometric studies

The catalytic oxidation of LD by a FCMCNPE was also studied by chronoamperometry. Chronoamperometric measurements of different concentrations of LD at FCMCNPE were done by setting the working electrode potential at 450 mV. In chronoamperometric studies, we have determined the diffusion coefficient, D, of LD. The experimental plots of I versus $t^{-1/2}$ with the best fits for different concentrations of LD were employed. The slopes of the resulting straight lines were then plotted versus the LD- concentrations, from whose slope and using the Cottrell equation [33]:

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

We calculated a diffusion coefficient of 1.95×10^{-6} cm^2 s^{-1} for LD.

The rate constant for the chemical reaction between LD and redox sites in FCMCNPE, k_h, can be evaluated by chronoamperometry according to the method of Galus [17]:

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \qquad (3)$$

Where I_C is the catalytic current of LD at the FCMCNPE , I_L the limited current in the absence of LD and t is the time elapsed (s). 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 versus $t^{1/2}$ plots (Fig. 4.A), k_h can be obtained for a given LD concentration. From the values of the slopes an average value of k_h was found to be $k_h = 4.54\times10^3$ M^{-1} s^{-1}. The value of k_h explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of LD at the surface of FCMCNPE. Finally, the heterogeneous rate constant of catalytic reaction was calculated as $k'_h = 8.4 \times 10^{-3}$ cm s^{-1}.

Double potential step chronocoloumetry, as well as other electrochemical methods, was also employed for the investigation of electrode processes at a FCMCNPE. Forward and backward potential step chronocoloumetry on the modified electrode in a blank buffer solution showed very symmetrical chronocolougrams. These had about an equal charge consumed for both oxidation and reduction of the Fc/Fc^+ redox system in the FCMCNPE. However, in the presence of LD, the charge value associated with forward chronocoloumetry was significantly greater than that observed for backward chronocoloumetry. This behavior is typical of that expected for electrocatalysis at chemically modified electrodes [33].

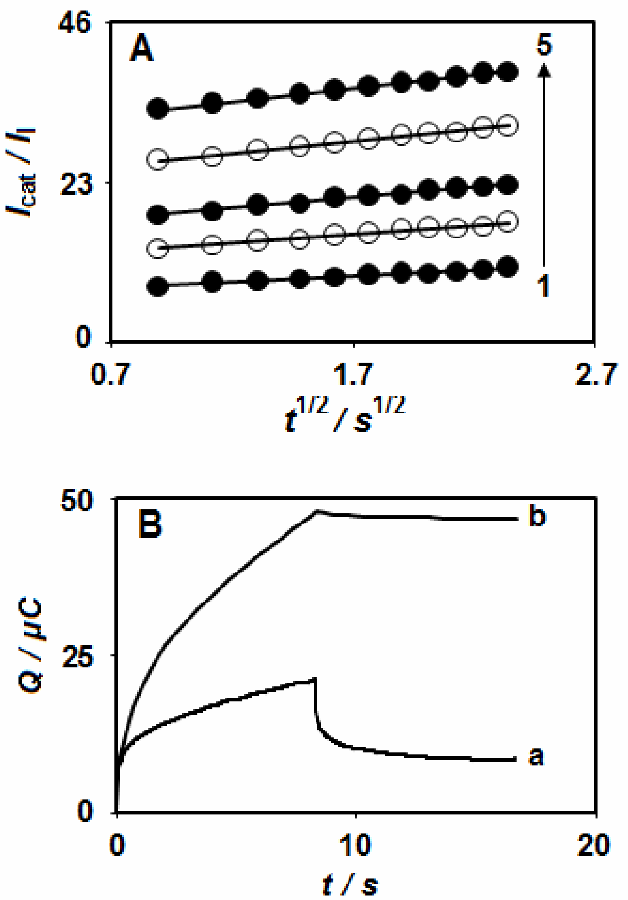

Figure 4. (A) Dependence of I_C/I_L on the $t^{1/2}$ driven from the chronoamperogram data. (B) FCMCNPE chronocolougrams in the absence (a) and presence of LD.

3.4. Calibration plot and limit of detection

Differential pulse voltammetry was used to determine the concentration of LD (Fig. 5A). Responses were linear with LD concentrations ranging from 2.0×10^{-6} to 5.0×10^{-4} M and a current sensitivity of 0.0318 μA/μM (Fig. 5B). The detection limit (3σ) was 1.2 μM.

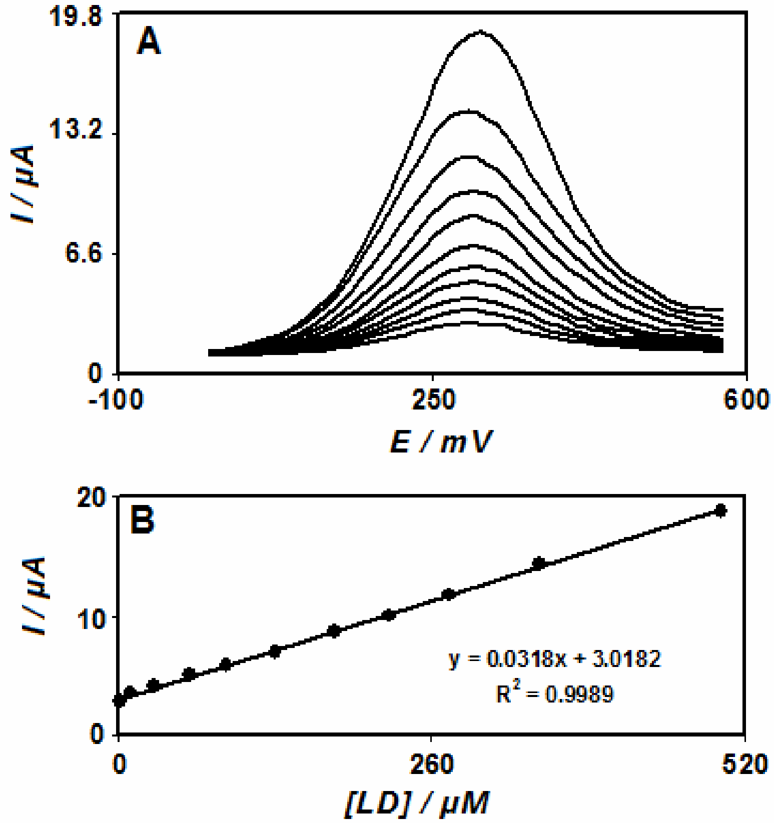

Figure 5. (A) Differential pulse voltammograms of the FCMCNPE in 0.1 M phosphate buffer solution (pH7.0) containing different concentrations of LD, from inner to outer correspond to 2, 10, 30, 60, 90, 130, 180, 225, 275, 350 and 500 μM of LD. (B) plots of the electrocatalytic peak current as a function of LD concentration.

3.5. Determination of LD real sample

To evaluate the applicability of the proposed method to real samples, it was applied to the determination of LD in urine sample. The LD contents were measured after sample preparation using the standard addition method. The results are given in Table 2.

Table 2. Determination of LD in urine

Sample No.	Added (μM)	Expected (μM)	Found (μM)	Recovery (%)	RSD (%)
1	0	---	<LOD	---	---
2	30	30	29.3	97.7	1.85
3	50	50	51.2	102.4	1.72

4	70	70	68.7	98.1	2.47
5	90	90	93.1	103.4	2.15

Result based on five replicate determinations per samples. Theoretical values for t=2.31 and F=6.39 (p=0.05)
LOD: Limit of detection

4. CONCLUSIONS

A carbon paste electrode modified with FC and carbon nanotubes has been fabricated and used for electrocatalytic determination of LD. The results demonstrated that the electrooxidation of LD at the surface of FCMCNPE occurs at a potential about 365 mV less positive than bare carbon paste electrode. The modified electrode has been shown to be promising for LD detection with many desirable properties including good reproducibility, high sensitivity, excellent catalytic activity, low detection limit and especially its antifouling properties towards LD and its oxidation products. Finally, this method was used for the determination of LD in urine sample using standard addition method.

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