

# Simultaneous determination of levodopa, NADH, and tryptophan using carbon paste electrode modified with carbon nanotubes and ferrocenedicarboxylic acid

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**Abstract** The redox response of a modified carbon nanotube paste electrode of ferrocenedicarboxylic acid was investigated. Cyclic voltammetry, differential pulse voltammetry, and chronoamperometry were used to investigate the electrochemical behavior of levodopa (LD) at modified electrode. Under the optimized conditions (pH 5.0), the modified electrode showed high electrocatalytic activity toward LD oxidation; the overpotential for the oxidation of LD was decreased by more than 190 mV, and the corresponding peak current increased significantly. Differential pulse voltammetric peak currents of LD increased linearly with its concentrations at the range of 0.04 to 1,100  $\mu\text{M}$ , and the detection limit ( $3\sigma$ ) was determined to be 12 nM. The diffusion coefficient ( $D = 9.2 \times 10^{-6} \text{cm}^2/\text{s}$ ) and transfer coefficient ( $\alpha=0.49$ ) of LD were also determined. Mixture of LD, NADH, and tryptophan (TRP) can be separated from one another by differential pulse voltammetry. These conditions are sufficient to allow determination of LD, NADH, and TRP both individually and simultaneously. The modified electrode showed good reproducibility, remarkable long-term stability, and especially good surface renewability by simple mechanical polishing. The results showed that this electrode could be used as an electrochemical sensor for determination of LD, NADH, and TRP in real samples such as urine and water samples.

**Keywords** Levodopa · NADH · Tryptophan · Carbon nanotube · Carbon paste electrode

## Introduction

Carbon nanotubes (CNTs) have captured worldwide researchers' interests since their discovery in 1991 [1]. Because of their small dimensions, high surface area, unique structures, ultrastrong mechanical properties, and high stability, carbon nanotubes hold the potential for a wide range of applications, such as supports for heterogeneous catalysts, especially in liquid-phase reactions. The subtle electronic properties suggest that carbon nanotubes have the ability to promote electron transfer reactions, indicating that they are good candidates for electrode in electrochemical reactions [2–5].

Carbon paste electrodes (CPEs), due to their ease of construction, renewability, and compatibility with various types of modifiers, have been widely used as a suitable matrix for preparation of modified electrodes. Further, they show rather low background current compared to the solid graphite or noble metal electrodes [6]. In recent years, application of the CNT-modified carbon paste electrodes showed considerable improvements in electrochemical behavior of biologically important compounds [7–10].

The unusual amino acid levodopa (3,4-dihydroxyphenylalanine, LD) is the precursor required by the brain to produce dopamine, a neurotransmitter (chemical messenger in the nervous system). People with Parkinson's disease have depleted levels of dopamine, and LD is used to increase dopamine in the brain, which reduces the symptoms of Parkinson's disease. Nevertheless, auto-oxidation of LD generates toxic metabolites, such as free radicals,

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semiquinones, and quinones. In vitro, LD is a powerful toxin that is lethal to the culture of neurons, and a few animal studies have shown that chronic LD may be toxic in vivo, too [11].

A variety of analytical methods have been developed in order to measure LD levels in different sample matrices, such as HPLC [12], spectrofluorimetry [13], circular dichroism [14], flow injection analysis [15], fluorescence spectrometry [16], photokinetic [17] and voltammetric [18–21] methods, etc.

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.

Dihyronicotinamide adenine dinucleotide (NADH) and its oxidized form (NAD<sup>+</sup>) are cofactors of more than 500 kinds of dehydrogenase enzymes widely used for the construction of electrochemical biosensors. However, the direct oxidation of NADH at conventional electrodes, such as carbon, gold, and platinum, is highly irreversible and only proceeds at considerable overpotentials owing to the sluggish charge transfer kinetics [22]. High overpotential also favors the formation of some inactive dimers that contaminate the electrode surface (electrode fouling). Both issues can be alleviated by incorporation of an electron mediator to shuttle electrons between NADH and the conducting substrate [23]. Various mediators [24–30] have been adopted for this purpose.

Tryptophan (TRP), a vital constituent of proteins and a precursor for biologically important molecules such as the neurotransmitter serotonin and the neurohormone melatonin, is an amino acid essential to humans and animals due to its physiological roles [31]. It is also indispensable in human nutrition for establishing and maintaining a positive nitrogen balance [32]. Unfortunately, TRP cannot be synthesized directly in human body and therefore must be taken from dietary, food products, and pharmaceutical formulas, in which TRP sometimes is added because the presence of TRP in vegetables is scarce. Nevertheless, when improperly metabolized, a waste product will be created in the brain to cause hallucinations and delusions [33]. Therefore, to establish simple, sensitive, and selective methods for TRP determination in food, pharmaceutical products, and living bodies especially for humans is very important.

For simple, sensitive, and less expensive detection of TRP, the methods based on electroanalysis might be the most notable due to its electrochemical activity [32, 34–37].

A practical mediator needs to have a low relative molar mass while being reversible, fast reacting, regenerated at

low potential, pH independent, stable in both oxidized and reduced forms, unreactive with oxygen, and nontoxic. Among the most successful mediators are those based on ferrocene and its derivatives that meet the above criteria. The importance of ferrocene and its derivatives as a mediator for electrooxidation processes has been known for several years [38–40].

Exogenous application of LD is conventionally used to equalize the striatal dopamine deficit in idiopathic Parkinson's disease. The stimulation of endogenous biosynthesis of LD via activation of tyrosine hydroxylase has been proposed as new therapeutic concept in Parkinson's disease. This may be achieved by exogenous supply with the reduced coenzyme NADH [41]. Also, certain individual amino acids are precursors to brain neurotransmitters and significantly ameliorate symptoms when given as dietary supplements. Tyrosine, phenylalanine, and TRP can all be blocked from absorption by LD, thereby becoming deficient.

Parkinson's disease patients treated with LD can manifest low serum TRP [42], and L-TRP therapy often helps them break through their depression [43].

To our knowledge, no study has reported the electrocatalytic and simultaneous determination of LD, NADH, and TRP by using ferrocenedicarboxylic-acid-modified carbon nanotube paste electrode (FCDMCNPE). Thus, in the present work, we described initially the preparation and suitability of a FCDMCNPE as a new electrode in the electrocatalysis and determination of LD in an aqueous buffer solution; then we evaluated the analytical performance of the modified electrode in quantification of LD in the presence of NADH and TRP. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of LD, NADH, and TRP in real samples, we examined this method for the voltammetric determination of LD, NADH, and TRP in urine and water samples.

## Experimental

### Apparatus and reagents

All the cyclic voltammetric measurements were performed using a BHP 2063<sup>+</sup> Electrochemical Analysis System, Behpajooh, Iran potentiostat/galvanostat coupled with a Pentium IV personal computer connected to a HP laser jet 6 L printer.

An Ag/AgCl/KCl 3 M, a platinum wire, and a FCDMCNPE were used as reference, auxiliary, and working electrodes, respectively. A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. Graphite powder, paraffin oil, and reagents were of

analytical grade from Merck. LD, NADH, and TRP were purchased from Merck. Multiwalled carbon nanotubes (purity of more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 0.5 to 200 nm were prepared from Nanostructured and Amorphous Materials (USA). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range 2.0–12.0.

#### Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by dissolving 0.01 g of ferrocenedicarboxylic (FCD) in diethyl 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 FCD-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 (approximately 3.4 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 was prepared in the same way without adding FCD and carbon nanotube to the mixture and was used for comparison purposes.

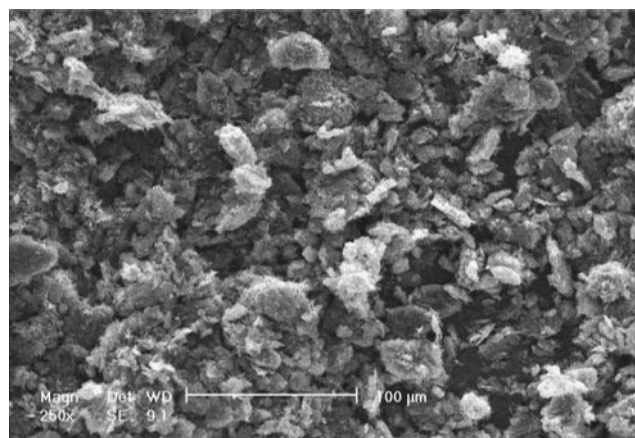
## Results and discussion

### SEM characterization

For study surface of modified electrode, we used SEM. Results show that, at a surface of modified electrode, mediator particle and carbon nanotubes packed and dispersed homogeneously (Fig. 1).

### Electrochemical behavior of FCDMCNPE

Cyclic voltammetry was employed for the investigation of the electrochemical properties of FCDMCNPE in a pure buffered aqueous solution (pH 5.0). The cyclic voltammogram exhibits an anodic and corresponding cathodic peaks with  $E_{pa}=0.50$  V and  $E_{pc}=0.38$  V vs. Ag/AgCl/KCl 3 M. The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc<sup>+</sup> redox couple with quasireversible behavior because the peak separation potential,  $\Delta E_p = (E_{pa} - E_{pc})$ , is greater than that 59/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



**Fig. 1** SEM image of FCDMCNPE

shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of Fc/Fc<sup>+</sup> in FCDMCNPE 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 FCDMCNPEs (Table 1). The calculated relative standard deviations (RSDs) for various parameters are 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.

### pH optimization of the solution

The electrochemical behavior of LD is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of Fc/Fc<sup>+</sup> redox couple are independent on pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of LD. Thus, we studied the electrochemical behavior of LD in 0.1 M phosphate-buffered solutions in different pH values (2.0 < pH < 12.0) at the surface of FCDMCNPE by cyclic voltammetry. It was found that the electrocatalytic oxidation of LD at the surface of FCDMCNPE was more favored under acidic conditions than in neutral medium. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms drawn at the surface of FCDMCNPE. The results show that the anodic peak potential of LD at the surface of FCDMCNPE was shifted to a less-positive potential. Also, the anodic peak current and the shifted potential value for electrooxidation of LD are high at an acidic pH. Thus, pH 5.0 was chosen as the optimum pH for electrocatalysis of LD oxidation at the surface of FCDMCNPE.

**Table 1** Cyclic voltammetric data obtained for constructed FCDMCNPE in 0.1 PBS (pH 5.0) at 20 mV s<sup>-1</sup>

$E_{pa}$ (V) <sup>a</sup>	$E_{pc}$ (V) <sup>a</sup>	$E_{1/2}$ (V) <sup>a</sup>	$\Delta E_{pa}$ (V) <sup>a</sup>	$I_{pa}$ ( $\mu$ A)	$I_{pc}$ ( $\mu$ A)	$\Gamma_a$ (mol cm <sup>-2</sup> )	$\Gamma_c$ (mol cm <sup>-2</sup> )
0.50 (0.68) <sup>b</sup>	0.38 (0.76) <sup>b</sup>	0.44 (0.62) <sup>b</sup>	0.12 (0.73) <sup>b</sup>	1.91 (1.94) <sup>b</sup>	0.81 (1.86) <sup>b</sup>	$2.37 \times 10^{-9}$ (2.92) <sup>b</sup>	$2.13 \times 10^{-9}$ (3.17) <sup>b</sup>

<sup>a</sup> Versus Ag/AgCl/KCl 3 M as reference electrode

<sup>b</sup> The values in parenthesis indicate the calculated RSD

### Electrochemistry of LD at FCDMCNPE

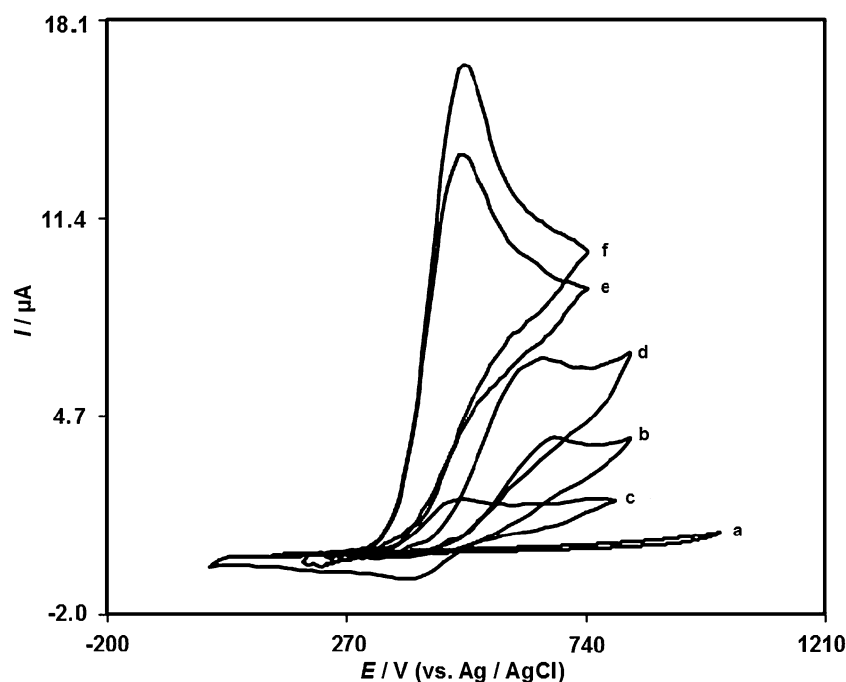
Figure 2 depicts the cyclic voltammetric responses from the electrochemical oxidation of 200  $\mu$ M LD at FCDMCNPE (curve f), FCD-modified CPE (curve e), CNPE (curve d), and bare CPE (curve a). As can be seen, the anodic peak potential for the oxidation of LD at FCDMCNPE (curve f) and FCDMCPE (curve e) is about 500 mV, while at the CNPE (curve d) peak potential is about 650 mV, and at the bare CPE (curve b) peak potential is about 690 mV for LD. From these results, it is concluded that the best electrocatalytic effect for LD oxidation is observed at FCDMCNPE (curve f). For example, the results are shown, in which the peak potential of LD oxidation at FCDMCNPE (curve f) shifted by about 150 and 190 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 FCDMCPE (curve e) and FCDMCNPE (curve f), there is a dramatic enhancement of the anodic peak current at FCDMCNPE relative to the value obtained at the FCDMCPE. In the other words, the data obtained clearly show that the combination of carbon nanotube and mediator (FCD)

definitely improve the characteristics of LD oxidation. The FCDMCNPE in 0.1 M phosphate buffer (pH 5.0), without LD in solution, exhibits a well-behaved redox reaction (curve c) upon the addition of 200  $\mu$ M LD; the anodic peak current of mediator was greatly increased, while the corresponding cathodic peak disappeared on the reverse scan of the potential (curve f). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes [44]. The reaction scheme would probably follow the mechanistic steps, in which LD can be oxidized by ferrocenium dicarboxylic acid ion produced in the surface of electrode:

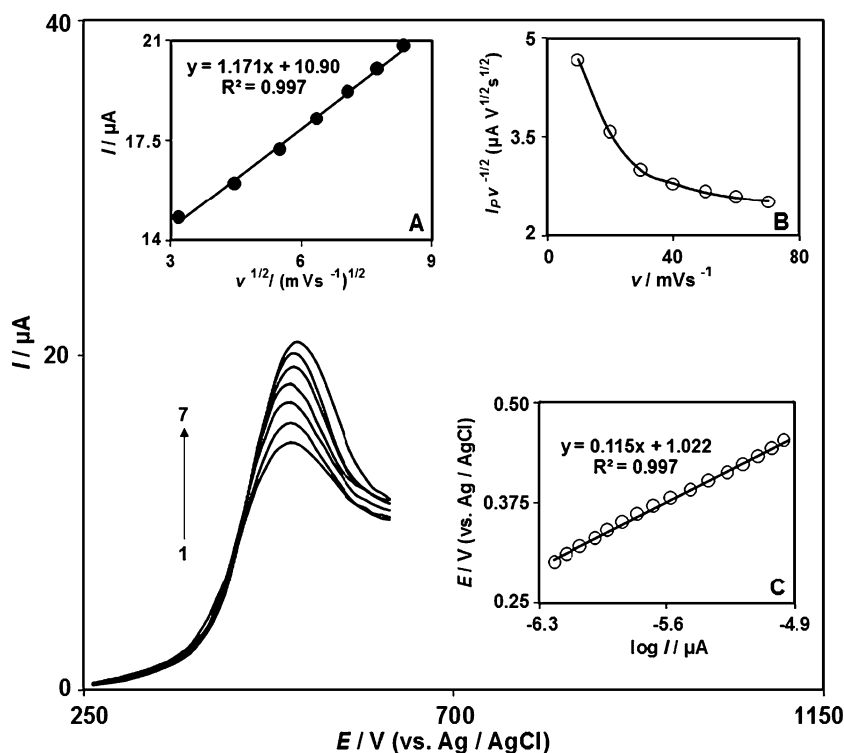


The effect of scan rate on the electrocatalytic oxidation of 200  $\mu$ M LD at the FCDMCNPE was investigated by liner sweep voltammetry (Fig. 3). The oxidation peak potential shifts with increasing scan rates towards a more positive potential, confirming the kinetic limitation of the

**Fig. 2** Cyclic voltammograms of (a) bare carbon paste electrode in buffer solution, (b) bare carbon paste electrode in the presence of 200  $\mu$ M LD, (c) carbon nanotube and FCD-modified electrode in buffer solution, (d) CNT-modified electrode in the presence of 200  $\mu$ M LD, (e) FCD-modified electrode in the presence of 200  $\mu$ M LD, and (f) carbon nanotube and FCD-modified electrode in the presence of 200  $\mu$ M LD. In all cases, scan rate is 20 mV s<sup>-1</sup>



**Fig. 3** Linear sweep voltammograms of the FCDMCNPE in the presence of 200 μM LD at various scan rates; the numbers 1–7 correspond to 10, 20, 30, 40, 50, 60, and 70 mVs<sup>-1</sup> scan rates, respectively, *Insets:* (a) The variation of the anodic peak currents vs.  $v^{1/2}$ . (b) Variation of the scan-rate-normalized current ( $I_p/v^{1/2}$ ) with scan rate. (c) Tafel plot derived from the rising part of voltammogram recorded at a scan rate of 20 mVs<sup>-1</sup>



electrochemical reaction. Also, a plot of peak height ( $I_p$ ) against square root of scan rate ( $v^{1/2}$ ), in the range of 10–70 mVs<sup>-1</sup>, was constructed (Fig. 3, a), which was found to be linear, suggesting that at sufficient overpotential the process is diffusion-controlled rather than surface-controlled. A plot of the sweep rate normalized current ( $I_p/v^{1/2}$ ) versus sweep rate (Fig. 3, b) exhibits the characteristic shape typical of an EC<sub>cat</sub> process [44].

Figure 3 (c) 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<sup>-1</sup>. This part of voltammogram, known as Tafel region [44], is affected by electron transfer kinetics between LD and FCD, 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 of 0.115 V per decade is obtained, indicating a one-electron transfer to be rate limiting assuming a transfer coefficient of  $\alpha=0.49$ .

**Chronoamperometric measurements**

The chronoamperometry as well as the other electrochemical methods was employed for the investigation of electrode processes at chemically modified electrodes. Chronoamperometric measurements of LD at FCDMCNPE were done by setting the working electrode potential at 600 mV for various concentrations of LD (Fig. 4). For an electroactive material (LD in this case) with a diffusion coefficient of  $D$ , the current for the electrochemical reaction (at a mass transport

limited rate) is described by the Cottrell equation [44]. Under diffusion control, a plot of  $I$  versus  $t^{-1/2}$  will be linear, and from the slope the value of  $D$  can be obtained (Fig. 4, inset a). The mean value of  $D$  was found to be  $9.2 \times 10^{-6}$  cm<sup>2</sup>/s.

Also, chronoamperometry can also be employed to evaluate the catalytic rate constant,  $k$ , for the reaction between LD and the FCDMCNPE according to the method of Galus [45]:

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

where  $t$  is the time elapsed and  $C_b$  is the bulk concentration of LD. The above equation can be used to calculate the rate constant of the catalytic process  $k$ . Based on the slope of the  $I_C/I_L$  versus  $t^{1/2}$  plot;  $k$  can be obtained for a given LD concentration. Such plots obtained from the chronoamperograms in Fig. 4 are shown in Fig. 4, inset b. From the values of the slopes, an average value of  $k$  was found to be  $k = 2.32 \times 10^3$  M<sup>-1</sup>s<sup>-1</sup>.

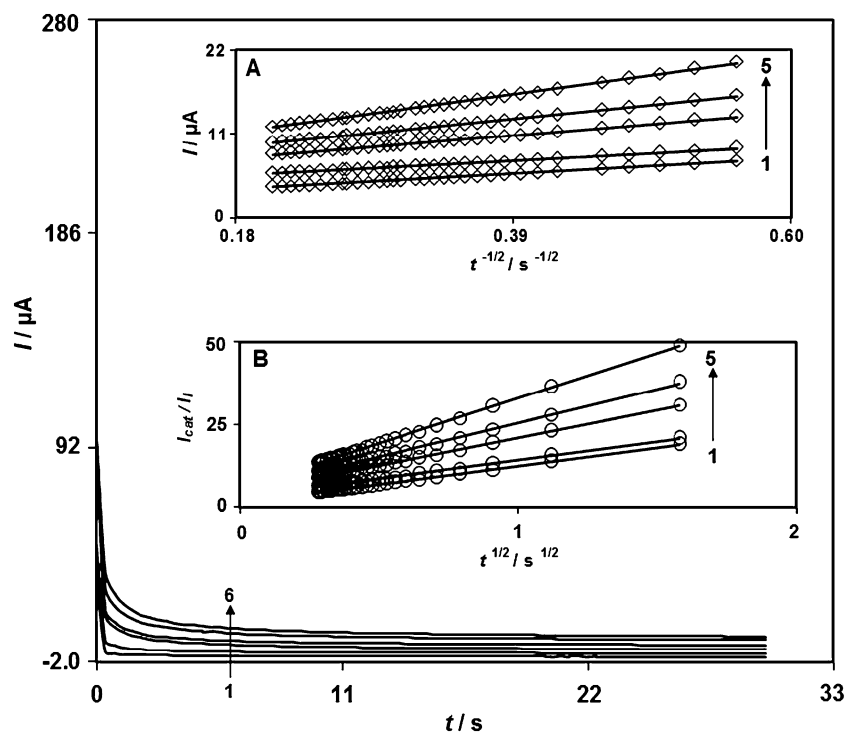
The value of  $k$  explains as well the sharp feature of the catalytic peak observed for catalytic oxidation of LD at the surface of FCDMCNPE. Finally, the heterogeneous rate constant ( $k'$ ) of catalytic reaction was calculated as  $k' = 5.49 \times 10^{-3}$  cms<sup>-1</sup>.

**Calibration plot and limit of detection**

Differential pulse voltammetry was used to determine the concentration of LD. Voltammograms clearly show that the



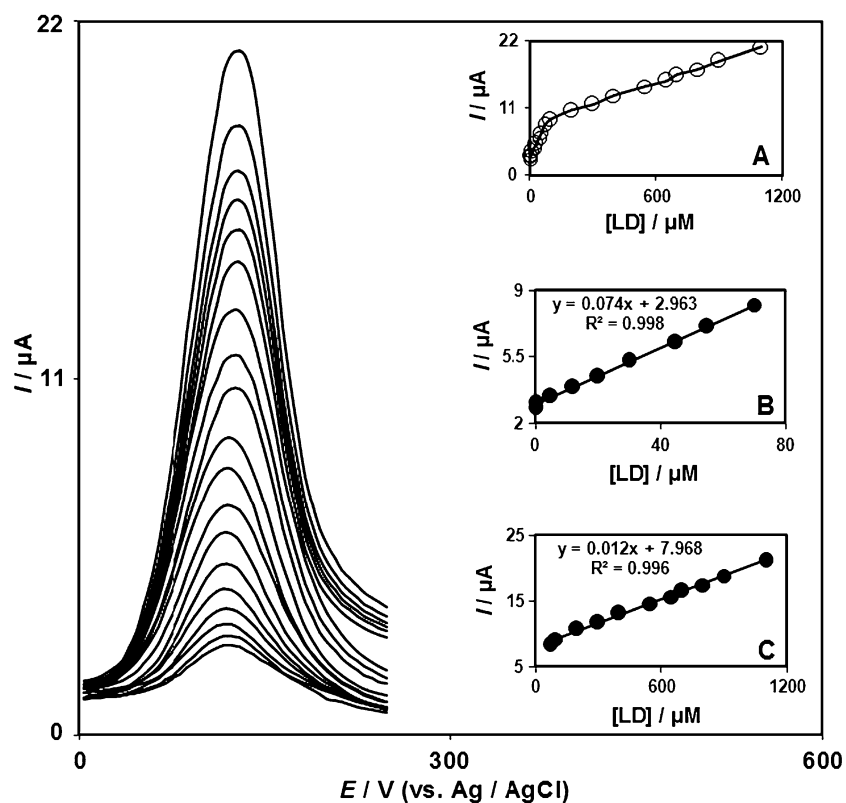
**Fig. 4** Chronoamperograms obtained at FCDMCNPE in 0.1 M phosphate buffer solution (pH 5.0) for different concentrations of LD. The numbers 1–6 correspond to 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM of LD. Insets: (a) plots of  $I$  vs.  $t^{-1/2}$  obtained from chronoamperograms 2–6. (b) Dependence of  $I_{\text{cat}}/I_1$  on  $t^{1/2}$  derived from the data of chronoamperograms



plot of peak current versus LD concentration is constituted of two linear segments with different slopes (slope  $0.074 \mu\text{A} \mu\text{M}^{-1}$  for first linear segment and  $0.012 \mu\text{A} \mu\text{M}^{-1}$  for second linear segment), corresponding to two different

ranges of substrate concentration, 0.04 to  $70.0 \mu\text{M}$  for first linear segment and  $70.0$  to  $1,100.0 \mu\text{M}$  for second linear segment (Fig. 5). The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation.

**Fig. 5** Differential pulse voltammograms of the FCDMCNPE in 0.1 M PBS (pH 5.0) containing different concentrations of LD, from inner to outer corresponds to 0.04, 0.1, 5, 12, 20, 30, 45, 55, 70, 100, 200, 300, 400, 550, 650, 700, 800, 900, and  $1,100 \mu\text{M}$  of LD. Insets: (a) plot of the electrocatalytic peak current as a function of LD concentration in the range of 0.04 to  $1,100.0 \mu\text{M}$ . (b) Plot of the electrocatalytic peak current as a function of LD concentration in the range of 0.04 to  $70.0 \mu\text{M}$  and (c)  $70.0$  to  $1,100 \mu\text{M}$



**Table 2** Comparison of the efficiency of some modified electrodes used in the electrocatalysis of LD

Electrode	Modifier	Method	LDR (M)	LOD (M)	Sensitivity ( $\mu\text{A}/\mu\text{M}$ )	Ref.
Glassy carbon	B-cyclodextrin-doped poly(2,5 diaminobenzenesulfonic acid)	Voltammetry	$1.0 \times 10^{-6}$ – $2.0 \times 10^{-4}$	$4.18 \times 10^{-7}$	0.925	18
Carbon paste	Dysprosium nanowire	Voltammetry	$1.0 \times 10^{-8}$ – $1.0 \times 10^{-6}$	$4.0 \times 10^{-9}$	0.754	19
Glassy carbon	Polypyrrole-doped with tiron	Voltammetry	$1.0 \times 10^{-6}$ – $1.0 \times 10^{-4}$	$1.0 \times 10^{-7}$	2.0	20
Graphite polyurethane	Oxovanadium-salen	Amperometry	$1.0 \times 10^{-6}$ – $1.0 \times 10^{-4}$	$8.0 \times 10^{-7}$	0.250	21
Pyrolytic graphite	Gold nanoparticle-functionalized carbon nanotubes	Voltammetry	$1.0 \times 10^{-7}$ – $1.5 \times 10^{-4}$	$5.0 \times 10^{-8}$	0.011	46
Carbon nanotube paste	Ferrocenedicarboxylic acid	Voltammetry	$4.0 \times 10^{-7}$ – $1.1 \times 10^{-3}$	$1.2 \times 10^{-8}$	0.074	This work

The detection limit ( $3\sigma$ ) for LD in the lower-range region was found to be 12 nM. The values for linear dynamic range, detection limit, and sensitivity are comparable to values reported by other researchers for the electrocatalytic oxidation of LD at the surface of chemically modified electrodes by other mediators previously (Table 2).

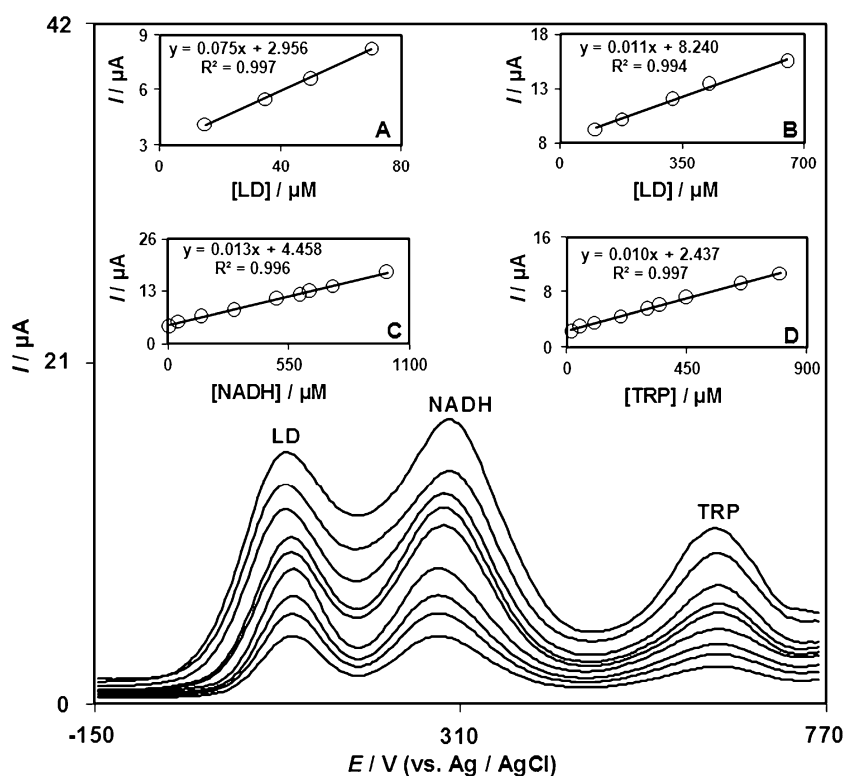
#### Simultaneous determination of LD, NADH, and TRP at FCDMCNPE

One of the main objectives of the present study was the development of a modified electrode capable of the elec-

trocatalytic oxidation of LD and separation of the electrochemical responses of LD, NADH, and TRP.

The utilization of the FCDMCNPE for the simultaneous determination of LD, NADH, and TRP was demonstrated by simultaneously changing the concentrations of LD, NADH, and TRP. The DP voltammetric results show three well-defined anodic peaks (Fig. 6), while the bare carbon paste electrode only gave an overlapped and broad oxidation peak. The sensitivity towards LD in the absence and presence of NADH and TRP was found to be  $0.074 \mu\text{A}\mu\text{M}^{-1}$  (absence of NADH and TRP) and  $0.075 \mu\text{A}\mu\text{M}^{-1}$  (presence of NADH and TRP). It is very

**Fig. 6** Differential pulse voltammograms of FCDMCNPE in 0.1 M PBS (pH 5.0) containing different concentrations of LD, NADH, and TRP (from inner to outer) mixed solutions of 15+10+15, 35+50+50, 50+150+100, 70+300+200, 100+500+300, 175+600+350, 320+650+450, 425+750+650, and 650+1,000+800, respectively, in which the first value is concentration of LD in micromolar and the second value is concentration of NADH in micromolar and the third value is TRP in micromolar. *Insets:* (a) and (b) plots of the peak currents as a function of LD, (c) NADH, and (d) TRP concentrations, respectively



interesting to note that the sensitivities of the modified electrode towards LD in the absence and presence of NADH and TRP are virtually the same, which indicates the fact that the oxidation processes of LD, NADH, and TRP, at the FCDMCNPE, are independent, and therefore simultaneous or independent measurements of the two analytes are possible without any interference. If the LD signal was affected by the NADH and TRP, the above-mentioned slopes would be different.

#### The repeatability and stability of FCDMCNPE

The long-term stability of the FCDMCNPE was tested over a 3-week period. The cyclic voltammetry of LD at the surface of FCDMCNPE after the modified electrode was stored in an atmosphere at room temperature shows that the oxidation peak potential of LD was unchanged and the anodic peak current was only decreased less than 1.95% 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 it in the presence of LD.

The cyclic voltammetry of LD at the surface of FCDMCNPE after 15 repetition cycles at a scan rate of  $20 \text{ mVs}^{-1}$  shows that the oxidation peak potential of LD was not changed and the anodic peak current was decreased by less than 2.8%. However, we regenerated the surface of FCDMCNPE before each experiment.

#### Interference studies

The influence of various substances as compounds potentially interfering with the determination of LD was studied

under optimum conditions with  $10 \mu\text{M}$  LD at pH 5.0. The potentially interfering substances were chosen from the group of substances commonly found with LD in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than  $\pm 5\%$  in the determination of LD. According to the results, neither a 700-fold excess of glucose, sucrose, lactose, fructose, or citric acid, nor a 500-fold excess of methanol, ethanol,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Al}^{3+}$ ,  $\text{NH}_4^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$ , or  $\text{F}^-$ , nor a 250-fold excess of alanine, methionine, phenylalanine, glycine, or folic acid (vitamin B<sub>9</sub>), nor a 100-fold excess of ascorbic acid, dopamine, and uric acid affected the selectivity. But, a 10-fold excess of CD interfered with the determination of LD.

#### Determination of LD, NADH, and TRP in urine and water samples

In order to evaluate the analytical applicability of the proposed method, it was also applied to the determination of LD, NADH, and TRP in urine samples. Known amounts of these drugs were added to the urine samples, and their concentrations were estimated with the proposed method. Simultaneous determination of drugs in the water sample was also performed. The sample was found to be free from LD, NADH, and TRP. Therefore, different amounts of LD, NADH, and TRP were spiked in the sample and analyzed by the proposed method. The results for determination of the three species in real samples are given in Table 3. Satisfactory recovery of the experimental results was found for LD, NADH, and TRP. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD).

**Table 3** The application of FCDMCNPE for simultaneous determination of LD, NADH, and TRP in urine and water samples

Sample	Spiked ( $\mu\text{M}$ )			Found ( $\mu\text{M}$ )			Recovery (%)			R.S.D. (%)		
	LD	NADH	TRP	LD	NADH	TRP	LD	NADH	TRP	LD	NADH	TRP
Urine	0	0	0	ND	ND	ND	–	–	–	–	–	–
	10	15	20	9.81	15.34	20.49	98.1	102.27	102.45	1.34	1.41	3.46
	15	20	25	15.44	20.21	24.31	102.9	101.05	97.24	3.32	2.27	1.16
	20	25	30	20.34	24.28	29.77	101.7	97.12	99.23	2.74	1.05	3.12
Well water	0	0	0	ND	ND	ND	–	–	–	–	–	–
	10	15	20	10.31	15.21	19.38	103.1	101.4	96.9	1.23	3.05	2.36
	15	20	25	15.28	19.47	25.49	101.87	97.35	101.96	1.23	1.96	3.27
	20	25	30	19.56	24.64	29.75	97.8	98.56	99.16	3.34	2.62	1.55
Tap water	0	0	0	ND	ND	ND	–	–	–	–	–	–
	10	15	20	9.91	14.81	20.67	99.1	98.73	103.35	2.25	3.16	1.97
	15	20	25	14.83	19.48	25.29	98.87	97.4	101.16	1.14	2.65	2.97
	20	25	30	20.32	25.89	29.61	101.6	103.56	98.7	2.33	3.14	1.37

ND not detected



## Conclusion

This work demonstrates the construction of a carbon nanotube paste electrode chemically modified by incorporation of FCD. The peak separation potential obtained for the  $FC/FC^+$  couple suggests that the couple can act as a quasireversible system in the carbon nanotube paste matrix. The carbon nanotube paste electrode modified with FCD was quite stable. This electrode is simple to prepare, and surface renewal is easy. The electrochemical behavior of FCD has been studied by cyclic voltammetry and chronoamperometry in both the absence and presence of LD. The results show that the oxidation of LD is catalyzed at pH 5.0, where the peak potential of LD is shifted by  $-190$  mV at the surface of the FCDMCNPE. The FCDMCNPE exhibits highly electrocatalytic activity for oxidation of LD, NADH, and TRP. The modified electrode displays improved selectivity for voltammetric measurement of LD, NADH, and TRP in solutions containing three analytes.

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