

A selective determination of levodopa in the presence of ascorbic acid and uric acid using a glassy carbon electrode modified with reduced graphene oxide

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Abstract A selective determination of levodopa (LD) in the presence of ascorbic acid (AA) and uric acid (UA) has been investigated at a glassy carbon electrode modified with reduced graphene oxide (rGO). The graphene oxide was synthesized chemically by Hummers method and characterized by energy-filtered transmission electron microscopy (EF-TEM). The reduced graphene oxide modified glassy carbon electrode (rGO/GCE) showed excellent electrochemical performance in the simultaneous electrochemical detection of LD, AA, and UA due to the unique properties of graphene, such as large surface area, facile electronic transport and high electrocatalytic activity. The redox characteristics of rGO/GCE were investigated with cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Well-resolved oxidation peak potentials, corresponding to the oxidation of AA, LD, and UA, were observed from their mixture solution at 0.098, 0.285, and 0.423 V, respectively. The rGO/GCE showed that LD can be detected without the interference of AA and UA. Under the optimized conditions, the oxidation peak current of LD is linear with the concentration of LD from 2.0 to 100 μM with the detection limit of 1.13 μM ($S/N = 3$). The present electrode system was also successfully applied to direct determination of LD in commercially available tablets and urine samples.

Keywords Levodopa · Ascorbic acid · Uric acid · Reduced graphene oxide · Electrochemical sensor · Voltammetry

1 Introduction

Parkinson's disease is a chronic neurodegenerative disease which causes progressive movement disorders when the substantia nigra of the mid-brain dies and fails to produce enough dopamine, a neurotransmitter (chemical messenger in the nervous system) [1]. This pathological state causes tremors, rigidity, slowness of movement, and loss of balance. Because dopamine is not able to penetrate the blood–brain barrier, it cannot be injected directly and be effectively available for the treatment of this severe disease [2]. Levodopa (3,4-dihydroxy-1-phenylalanine), one of the catecholamines and an essential precursor of dopamine, was introduced in the 1960s for the treatment of Parkinson's disease [3]. This catecholamine drug, in contrast to dopamine, is able to penetrate the blood–brain barrier and is metabolized by an enzymatic reaction (dopa-decarboxylase) to dopamine, increasing the concentration of dopamine in the brain [4]. However, the enzymatic metabolization of LD also occurs in the peripheral system, producing various side effects, such as gastritis, paranoia, vomiting, and dyskinesia, related to the increase of systemic dopamine [5, 6]. Some studies have shown that LD is a powerful toxin that is fatal to the culture of neurons *in vitro*, and a few animal studies have shown that chronic LD may also be toxic *in vivo* [7]. Therefore, development of a simple, economical, and precise analytical method for the determination of LD is important for analytical applications in pharmaceutical preparations.

In order to support the evaluation of LD in pharmaceutical formulations and biologic fluids, many techniques

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including spectrophotometry [8–10], chemiluminescence [11, 12], high-performance liquid chromatography [13–15], and ^1H NMR [16] have been developed. Nevertheless, each technique has various disadvantages associated with complicated and time-consuming pretreatments, expensive equipment, cost, selectivity, the use of organic solvents, and long analysis time. Compared with these methods, electrochemical techniques are more appropriate for real-time detection because of their simplicity, low cost, availability, high sensitivity, accuracy, and rapidity in the determination of biologically important substances.

As catecholamine compounds such as LD are electroactive, they can be oxidized and detected using an electrochemical technique [17]. It is unfortunate that the electrochemical oxidation of LD is sluggish due to slow electron transfer with high overpotential at most unmodified solid electrodes. There are some papers on the electrochemical determination of LD in pharmaceutical formulations using the modified electrodes [18–22]. The main problem involved in the measurement of LD *in vivo* is the interference of large amounts of substances such as ascorbic acid (AA) and uric acid (UA). Furthermore, at most solid electrodes, AA and UA are oxidized at a potential close to that of LD, resulting in an overlapping voltammetric response [23]. To resolve these problems, many chemically modified electrodes have been reported for LD determination [24, 25].

Graphene is a monolayer sheet of sp^2 bonded carbon atom with a two-dimensional (2D) honeycomb lattice. It has shown many outstanding characteristics, including a large specific surface area, high mobility of charge carriers, unusual transport performance, high mechanical strength, and significantly high thermal conductivity [26–28]. These distinctive properties attracted enormous attention from fundamental research because of its many possible applications. Graphene has been used to fabricate a new generation of electrodes for electrochemical research due to its great electrocatalytic activity toward some important biomolecules [29–34].

In this study, a simple and highly selective reduced graphene oxide (rGO) modified glassy carbon electrode was prepared using rGO sheets synthesized by a redox method. This electrochemical sensor was used for investigating the electrochemical behavior of LD at the surface of the GCE modified with rGO. Selective determination of LD by this sensor in the presence of high concentrations of AA and UA was demonstrated. It was found that the modified electrode not only exhibited strong electrocatalytic activity for oxidation of LD, AA, and UA, but also separated their voltammetric responses into three well-defined peaks. This sensor showed good selectivity and sensitivity in determination of LD and has been successfully used to measure the concentration of LD in pharmaceutical and biologic samples.

2 Experimental

2.1 Chemicals and reagents

Graphite powder (400 mesh), ammonia (25 wt % in H_2O), hydrogen peroxide (H_2O_2 , 30 wt % in H_2O), hydrazine ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 64–65 % in H_2O), uric acid (UA), and ascorbic acid (AA) were obtained from Sigma-Aldrich (USA). Levodopa (LD) was obtained from Tokyo Chemical Industry Co. (Japan). 0.1 M phosphate buffer solution (PBS) was prepared by mixing dipotassium hydrogen phosphate (K_2HPO_4) and monopotassium phosphate (KH_2PO_4), both of which were obtained from Junsei Chemical Co. (Japan). A commercial pharmaceutical tablet for LD determination was purchased from Merck Sharp & Dohme (Australia). All other chemicals were of analytical reagent grades and used without further purification. All solutions were prepared with deionized water obtained from an ultrapure water purification system (LabTech, Korea) with a resistivity of not less than $18.2 \text{ M}\Omega \text{ cm}$. All measurements were carried out at room temperature.

2.2 Apparatus

The electrochemical experiments were conducted using a CHI Model 842B (C.H. Instruments, Inc., USA) with a conventional three-electrode cell. The working electrode was a bare or modified glassy carbon electrode (GCE, 3 mm in diameter). Counter and reference electrodes were used by a platinum wire and a Ag/AgCl electrode filled with 3 M KCl, respectively. A transmission electron microscope (TEM) image was obtained using a LIBRA 120 plus Energy-Filtering TEM (Carl Zeiss, Germany). FT-IR spectra were recorded with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer in the range of $400\text{--}4,000 \text{ cm}^{-1}$ using a KBr pellet. All ultrasonic cleaning was done using a US-2510 Ultrasonic Cleaner (Branson, USA).

2.3 Synthesis of the reduced graphene oxide (rGO)

Graphene oxide (GO) was synthesized by a modified Hummers method [35]. Graphite powder (0.5 g) was put into a mixture of concentrated H_2SO_4 containing potassium peroxodisulfate (0.25 g) and phosphorus pentoxide (0.25 g). The mixture solution was heated to $80 \text{ }^\circ\text{C}$ and kept stirring for 6 h using an oil bath. Followed by the addition of 50 mL of water, the mixture was filtered using a $0.2 \text{ }\mu\text{m}$ Nylon film and washed with water several times. The filtrate was dried at $40 \text{ }^\circ\text{C}$ for 24 h. In order to produce GO, sulfuric acid and potassium permanganate were added to pre-oxidized graphite in an ice bath. After adding 23 mL of water, the mixture was stirred at $90 \text{ }^\circ\text{C}$ for 30 min. Then another 70 mL of water and 1.25 mL of 30 % H_2O_2 were

added to the mixture. The powder of GO was obtained by filtration and drying in an oven at 40 °C overnight. Graphene oxide powder was dispersed homogeneously in water by ultrasonication for 1 h to obtain a colloidal graphene oxide solution of 1 mg mL⁻¹. Reduction of 10 mL of GO dispersion was carried out by adding 7 μL of hydrazine and some amounts of ammonia necessarily to adjust the pH to 8.5. The solution was then kept stirring at 90 °C for 1 h. The final dispersion was filtered, washed, and dried in an oven at 40 °C overnight to obtain black and hydrophobic powder of rGO.

2.4 Preparation of reduced graphene oxide modified glassy carbon electrode (rGO/GCE)

Five mg of rGO was dispersed in 10 mL of water with ultrasonication for 1 h to form a black suspension. Prior to the modification, the bare glassy carbon electrode was typically polished first with a 0.3 μm wet alumina powder slurry and then a 0.05 μm wet alumina powder slurry to produce a mirror-like surface. It was then rinsed with water and ultrasonicated with 50 % nitric acid, ethanol, and water, each for 5 min, and dried under N₂. The rGO/GCE was prepared by dropping 4 μL of rGO suspension on the surface of a glassy carbon electrode. Before being used for experiments, the electrode was put in air at room temperature for some time in order to evaporate the solvent naturally.

2.5 Preparation of LD tablets and urine samples

Five tablets (containing 100 mg of levodopa each) were weighed and finely ground to powder using a mortar and pestle. The levodopa (449.6 mg of the powder from the previous step) was then transferred into a 100 mL of flask and dissolved into 20 mL of 1 % acetic acid. Next, the solution was diluted to a final volume of 100 mL with 0.1 M PBS solution (pH 7.0). After filtration the final solution was analyzed using the rGO modified GCE. A fresh urine sample was filtered and diluted 50 times using a 0.1 M PBS of pH 7.0 without any further treatment. The diluted urine sample was spiked with different amounts of LD, and their concentrations were measured using the DPV method.

3 Results and discussion

3.1 Characterization of rGO/GCE

The successful synthesis of rGO was confirmed by energy-filtered TEM. Figure 1 shows a typical TEM image of an rGO film, which reveals its rippled and crumbled layer planar sheet-like morphology. The TEM image indicates that the rGO nanosheets have a large surface area and

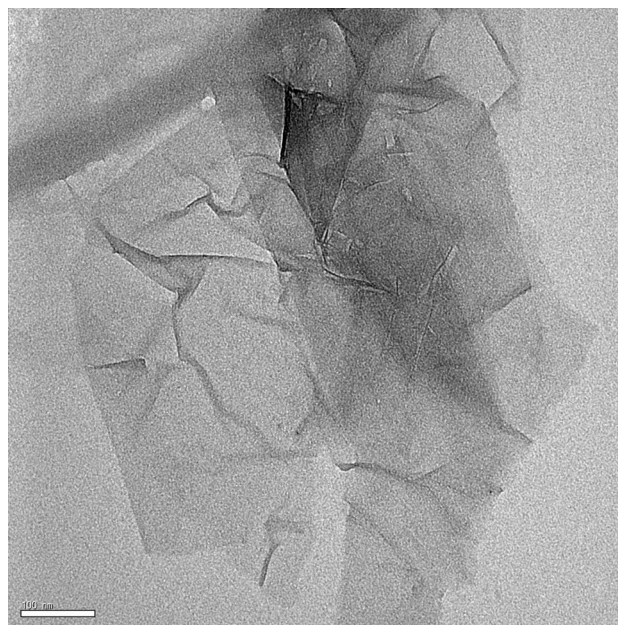


Fig. 1 EF-TEM image of reduced graphene oxide (rGO) dispersed in water. Length of the scale bar is 100 nm

length up to a few hundred nanometers, demonstrating that the exfoliation of graphene oxide down to an rGO sheet was achieved under our experimental conditions. The FT-IR spectra of rGO, GO, and graphite are shown in Fig. S1. The FT-IR spectrum of GO (Fig. S1b) is typical for GO and consistent with a previous report [36], while the absorption peaks at 3440, 1720, 1627, 1400, 1220, and 1053 cm⁻¹ are attributed to the O–H, C=O in COOH, aromatic C=C, carboxy C–O, epoxy C–O, and alkoxy C–O stretching modes, respectively. After a reduction by hydrazine, most of the oxygen-containing groups in the GO have been eliminated, while the C=C(1,627 cm⁻¹) remains in rGO (Fig. S1a). Potassium ferricyanide solution was first used to evaluate the electrochemical behavior of rGO/GCE. Figure 2 shows the cyclic voltammogram for the two different electrodes in 5 mM K₃Fe(CN)₆ in 0.1 M KCl. Compared with bare GCE, rGO/GCE typically shows an increase in redox peak current of Fe(CN)₆^{3-/4-} and a decrease in the peak-to-peak separation (ΔE_p). The observed ΔE_p values of bare and modified GCE are 172 and 83 mV, respectively. This clearly demonstrates that the transfer of electrons on the surface of rGO is much easier and more facile than on bare GCE. These electrochemical phenomena might be due to large surface area and high electric conductivity of rGO nanosheets.

3.2 Effect of concentration of rGO suspension

To study the effect of the amount of rGO coated on GCE, the modified electrode was prepared at different concentrations

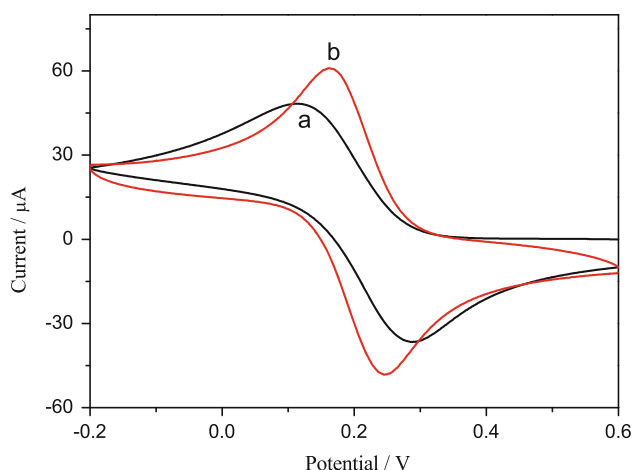


Fig. 2 CVs obtained at bare GCE (a) and rGO/GCE (b) in 0.1 M KCl containing 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ at scan rate of 100 mV s^{-1}

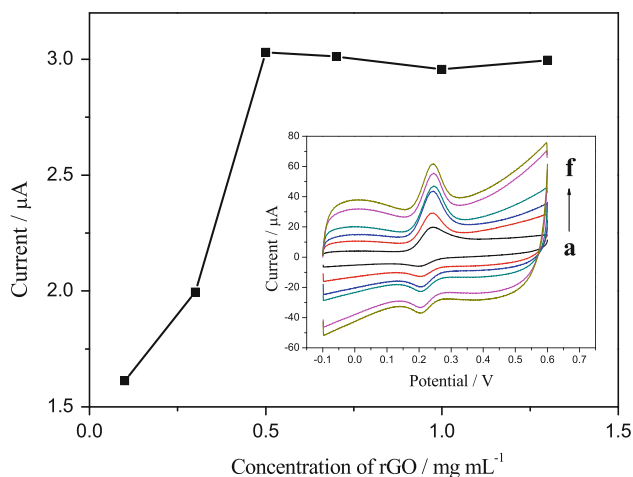


Fig. 3 Plot of I_{pa} versus the concentrations of rGO suspension. *Inset* CVs in 0.1 M PBS (pH 7.0) containing 0.3 mM LD at rGO/GCE coated with different concentrations of rGO suspension from 0.1 to 1.3 mg mL^{-1} at scan rate of 100 mV s^{-1} . The concentrations of rGO suspension: 0.1 mg mL^{-1} (a), 0.3 mg mL^{-1} (b), 0.5 mg mL^{-1} (c), 0.7 mg mL^{-1} (d), 1.0 mg mL^{-1} (e), 1.3 mg mL^{-1} (f)

of rGO suspensions of from 0.1 to 1.3 mg mL^{-1} . The CV experiment was performed in $3.0 \times 10^{-3} \text{ mol L}^{-1}$ of LD in 0.1 M PBS (pH 7.0) at a scan rate of 100 mV s^{-1} . As shown in Fig. 3, the oxidation peak current of LD increased with the increasing concentration of rGO suspension from 0.1 to 0.5 mg mL^{-1} and reached maximum current response at 0.5 mg mL^{-1} . Further increase of the concentration of rGO caused no significant increase in the current response. This phenomenon is probably due to the saturated active surface area in the excessive casting of rGO onto bare GCE surface, and no change is observed when increasing the concentration of rGO. Therefore, a concentration of 0.5 mg mL^{-1} is chosen as the optimum concentration of rGO suspension.

3.3 Effect of scan rate

The effect of scan rate on the voltammetric response of rGO/GCE in 0.1 M PBS (pH 7.0) containing $3.0 \times 10^{-3} \text{ mol L}^{-1}$ of LD was investigated. As shown in Fig. S2, the anodic peak currents of LD increased with an increase in the scan rate from 20 to 320 mV/s . The redox peak current shows a good linear relationship with the square root of the scan rate. The regression equation is $I_{\text{pa}} (\mu\text{A}) = -3.017 + 2.956 v^{1/2}$ with a correlation coefficient of 0.999. This result strongly suggests that the LD oxidation is under diffusion control process at the modified electrode. The peak potential separation increased with increasing scan rate, indicating that the redox reaction of LD is dependent on the scan rate. This fact confirmed that the electrochemical reaction of LD is not enough fast to reach equilibrium, resulting in a quasi-reversible process.

3.4 Effect of buffer solution pH

In order to optimize the buffer pH condition of detecting LD at rGO/GCE, as shown in Fig. 4, the redox peak potentials of LD were investigated over a pH range of 2.0–9.0 in a phosphate buffer solution. The oxidation peak current increased until the pH value reached 7.0 and then decreased. The maximum peak current was obtained at a pH of 7.0. Therefore, we have chosen 7.0 as the optimum pH value for the electrochemical detection of LD. It is interesting to note that electrochemically reversible behavior with a ratio of $I_{\text{pa}}/I_{\text{pc}}$ of nearly one is observed in the acidic pH region for the LD redox reaction. However, the cathodic peak current rapidly decreases when the pH value is higher than 7.0. This could be due to a following chemical reaction in which the deprotonated amine group of the oxidized levodopa molecule acts as a nucleophile, resulting in a 1,4-addition (Michael) reaction which has been previously reported for the electrochemical oxidation of epinephrine and dopamine [24]. As shown in inset of Fig. 4, the redox peak potential, $E_{\text{p}} = (E_{\text{pa}} + E_{\text{pc}})/2$, of LD negatively shifts with the increasing pH value of solution. The linear regression of the equation is $E_{\text{p}}(\text{V}) = -0.0547 \text{ pH} + 0.638$ with a correlation coefficient of 0.995 for LD. The slope of E_{p} versus pH is close to the theoretical value of 58.5 mV/pH, indicating that a two protons and two electrons process takes part in the redox reaction of LD at rGO/GCE [37].

3.5 Electrochemical behavior of LD at rGO/GCE

The electrochemical behavior of LD was investigated with cyclic voltammetry in 0.1 M PBS (pH 7.0). Figure 5c shows that a weak and broad response at the very positive potential for LD oxidation is observed at ca. 0.48 V on bare GCE, revealing that the electrode process is sluggish and substantially irreversible. On the other hand, using rGO/GCE, a well-

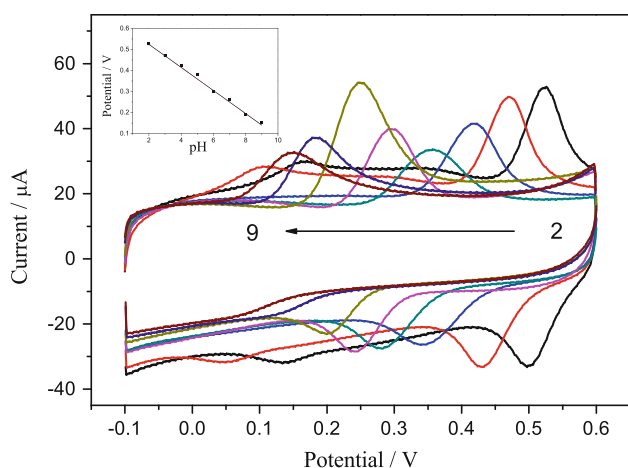


Fig. 4 CVs of 0.3 mM LD at rGO/GCE in 0.1 M PBS (pH 7.0) with different pH value from 2.0 to 9.0 at scan rate of 100 mV s^{-1} . *Inset* Plot of E_p versus pH

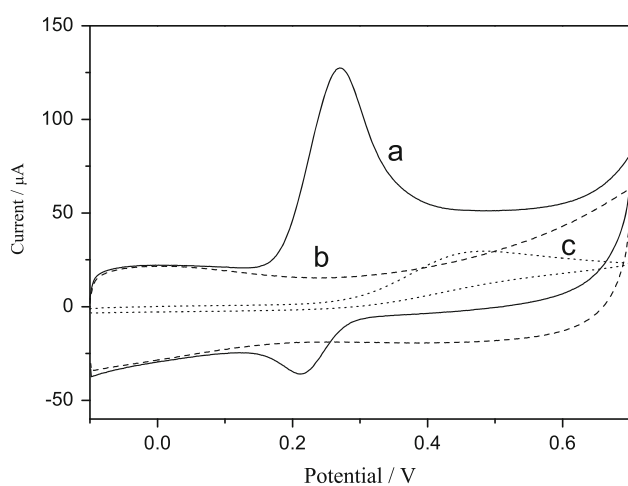


Fig. 5 CVs of rGO/GCE in the presence (a) and absence (b) of 1 mM LD and bare GCE (c) in the presence of 1 mM LD in 0.1 M PBS (pH 7.0) at scan rate of 100 mV s^{-1}

defined and sharp anodic peak of LD (shown in Fig. 5a) is observed at 0.27 V, indicating that the modified GCE can effectively decrease the oxidation potential of LD by 0.21 V with a peak-to-peak separation of about 57 mV. Furthermore, the oxidation peak current of LD at rGO/GCE is enhanced greatly and is approximately five times higher than that of bare GCE. These results confirm that the modified electrode not only improves electrochemical reactivity toward the oxidation of LD, but also that it can be applied as a sensitive voltammetric tool for the selective determination of LD.

3.6 Electrocatalytic oxidation of AA, LD, and UA

In the oxidative determination of LD by electrochemical methods, AA and UA are the major hampering species. This is primarily because AA and UA can be oxidized at

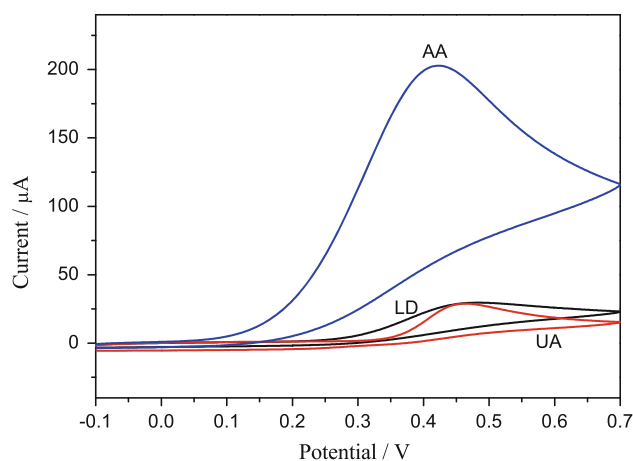


Fig. 6 CVs of bare GCE in 0.1 M PBS (pH 7.0) containing 10 mM AA, 1 mM LD, and 1 mM UA at scan rate of 100 mV s^{-1}

almost the same potential as LD, and since concentrations of AA and UA are relatively higher in biologic samples than that of LD, this can result in serious interference with the determination of LD [38]. Thus, it is important to investigate the selective determination of LD in the presence of AA and UA. Figs. 6 and S3 show typical voltammetric responses of bare GCE and rGO/GCE toward AA, LD, and UA. As can be seen in Fig. 6, AA, LD, and UA at bare GCE show irreversible oxidation peaks at 0.420, 0.480, and 0.463 V, respectively. However, for rGO/GCE (shown in Fig. S3), the anodic oxidation peaks of AA, LD, and UA appear at about 0.102, 0.270, and 0.424 V, respectively. Substantial negative shifts of AA, LD, and UA oxidation peak potentials are observed, which is probably due to the electrocatalytic activity of reduced graphene oxide. The peak potential differences between AA and LD and between UA and LD are about 168 and 154 mV, respectively. These differences are large enough to distinguish LD from AA and UA. In addition, the oxidation peak currents of LD, AA, and UA at the modified GCE are greatly improved compared with those at bare GCE, being approximately 7, 2, and 5 times higher, respectively (shown in Fig. 6). This may be due to the increased electrode surface active area of rGO/GCE.

3.7 Selective discrimination of LD in the mixture of AA and UA

In order to ascertain the simultaneous determination of LD, AA, and UA, cyclic voltammograms were recorded by bare GCE and rGO/GCE in 0.1 M PBS solution (pH 7.0) containing the mixture of LD, AA, and UA. As shown in the inset of Fig. 7a, a completely overlapped anodic peak is observed for bare GCE at around 0.438 V. In contrast, curve (a) in Fig. 7a shows that the modified GCE can

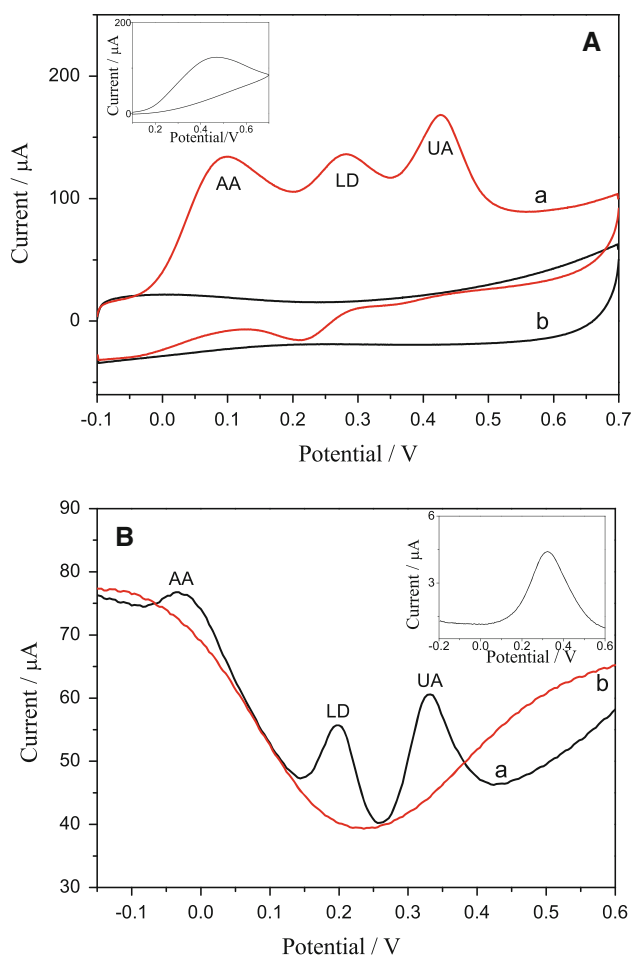


Fig. 7 **a** CVs of rGO/GCE in the presence (*a*) and absence (*b*) of 5 mM AA, 0.5 mM LD, and 0.3 mM UA in 0.1 M PBS (pH 7.0) at scan rate of 100 mV s^{-1} . *Inset* CV of bare GCE in the same mixture solution as described above. **b** DPV of rGO/GCE in the presence (*a*) and absence (*b*) of 500 μM AA, 20 μM LD, and 20 μM UA in 0.1 M PBS (pH 7.0). *Inset* DPV of bare GCE in the same mixture solution as described above

effectively distinguish the merged voltammetric peak into three well-resolved oxidation peaks at potentials around 0.098, 0.285, and 0.423 V for AA, LD, and UA, respectively. Similar peak resolution was also observed (Fig. 7b) when differential pulse voltammetry was used with the electrodes in 0.1 M PBS solution (pH 7.0) containing the mixture of AA, LD, and UA. The differential pulse voltammetric response at bare GCE (shown in the inset of Fig. 7b) was observed as only one weak and broad peak, indicating that the simultaneous determination of the three molecules is not possible at bare GCE. However, as was found for the modified GCE with the cyclic voltammogram in Fig. 7b, curve (a) of Fig. 7b shows that three well-defined differential pulse voltammetric peaks for AA, LD, and UA were recorded at 0.02, 0.208, and 0.336 V, respectively. The observed large separations of the peak potentials allow selective determination of LD in the

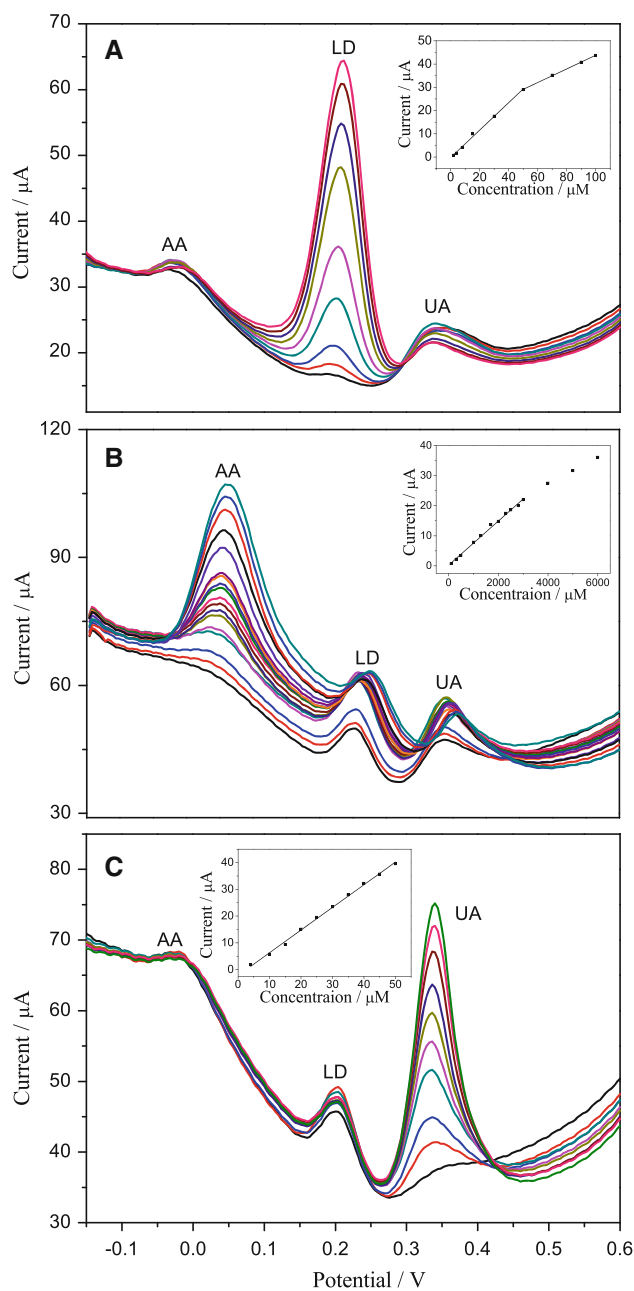


Fig. 8 **a** DPVs of rGO/GCE in the presence of 500 and 10 μM UA in 0.1 M PBS (pH 7.0) with different concentrations of LD. **b** DPVs of rGO/GCE in the presence of 10 μM LD and 10 μM UA in 0.1 M PBS (pH 7.0) with different concentrations of AA. **c** DPVs of rGO/GCE in the presence of 500 μM AA and 10 μM LD in 0.1 M PBS (pH 7.0) with different concentrations of UA

presence of AA and UA. Similar electrochemical behaviors have been previously reported for the selective determination of LD on the surface of glassy carbon electrodes modified with several types of polymer composite-incorporating carbon nanotubes [19, 24, 42], single-wall nanotubes [40], and multi-wall carbon nanotube composites containing metal hydroxide nanoparticles [41]. These electrochemical results may be attributed to the outstanding

Table 1 Comparison of analytical performance using different modified electrodes

Electrode	Method	Linear range (μM)	Limit of detection (μM)	Reference
CoHCF-LMC/GCE	DPV	0.1–1,900	0.017	18
CNT/chitosan/GCE	Chronoamperometry	1–360	0.86	19
AuNP-CNT/PGE	DPV	0.1–150	0.5	23
ppy/CNT/GCE	DPV	1–100	0.1	24
Q/fMWCNT/MGCE	DPV	0.90–85.0	0.381	25
SWNT/GCE	DPV	0.5–20	0.3	40
MWCNTs/NHNP _s /GCE	DPV	1–672	0.076	41
MWCNTs-CHT/GCE	DPV	2.0–220.0	0.6	42
PAR/GCE	DPV	25–400	6	43
EBNBH/CNPE	DPV	0.2–700	0.094	44
Nafion/Co(OH) ₂ -MWCNTs/CILE	DPV	0.25–225	0.12	45
rGO/GCE	DPV	2–100	1.13	This work

CoHCF-LMC cobalt hexacyanoferrate/large-mesopore; *PGE* pyrolytic graphite electrode; *ppy* poly-pyrrole; *Q/fMWCNT/MGCE* quercetin at a multi-walled carbon nanotubes modified glassy carbon electrode; *SWNT* single-wall carbon nanotube; *MWCNT* multiwall carbon nanotube; *NHNP_s* nickel hydroxide nanoparticles; *CHI* chitosan; *PAR*: poly(4-(2-pyridylazo)-resorcinol); *EBNBH* 2, 2'-[1,2-ethanediybis(nitrioloethylidene)]-bis-hydroquinone; *CNPE* carbon nanotube paste electrode; *CILE* carbon ionic liquid electrode

Table 2 Electrochemical determination of LD in commercial tablets

Sample no.	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
1	8	7.71	96.39	2.11
2	16	14.7	98.02	2.2
3	32	29.51	92.22	2.77

Table 3 Electrochemical determination of LD in human urine samples

	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
Urine	0	ND	–	–
	10	10.06	100.63	1.76
	15	15.16	100.98	1.75
	20	18.26	91.32	1.64

electronic conductivity, chemical stability, and high specific surface area of nanomaterials such as reduced graphene oxide and carbon nanotube.

3.8 Determination of LD in the presence of AA and UA by DPV

The quantitative determination of LD was carried out with DPV using rGO/GCE. DPV is commonly used for the determination of catecholamine compounds because of its high sensitivity and resolution, which is due to the small contribution of charging current to the background current [39]. In the electrochemical measurements, the concentration of LD was continuously increased with the successive addition of a standard solution, while the concentrations of the other interfering species remained constant. Figure 8a shows that

the oxidation peak current of LD increases with the increasing amount of LD. In the inset of Fig. 8a, the plot of peak current vs. LD concentration consists of two linear segments with different slopes. In the lower range region the anodic peak current of LD increases linearly with increasing the amount of LD from 2 to 50 μM in the mixture solution. The calibration equation is $I_{p, LD} (\mu A) = -0.22209 + 0.59059 C_{LD} (\mu M)$ with a correlation coefficient of 0.995. The detection limit for LD was found to be 1.13 μM with a signal-to-noise ratio of three. The electroanalytical performance characteristics of some electrodes using other modification methods to detect LD are summarized in Table 1. To investigate the interference of AA and UA in the accurate determination of LD, increasing additions of AA and UA are added to a buffer solution containing 10 μM LD. As we can see in Fig. 8b and 8c, while the oxidation current of AA and UA increase, that of LD was kept nearly constant. The results demonstrate that the coexistence of AA and UA has no influence on LD determination. Thus, rGO/GCE fabricated from a simple coating of reduced graphene oxide onto a glassy carbon electrode surface seems to be useful for developing a voltammetric sensor for the detection of LD in the mixture solution.

3.9 Real sample analysis

3.9.1 Determination of LD in commercial tablets

In order to evaluate the analytical applicability of a reduced graphene oxide modified GCE, the electrode was successfully applied to the direct determination of LD content of a pharmaceutical tablet (SINEMET). The DPV method was carried out with the addition of known amounts of

commercial tablet samples in a phosphate buffer solution. The results are summarized in Table 2. The recovery of LD by the proposed analytical method for a spiked tablet is from 92.22 to 98.02 %. The RSD is less than 2.77 % for tablet samples. These results for tablet samples are in good agreement with the labeled value.

3.9.2 Determination of LD in human urine

The proposed electrode was also applied to the determination of LD in urine samples at optimum conditions using the DPV method. LD was not found in urine samples, therefore, samples were spiked with different amounts of LD and were analyzed using the reduced graphene oxide modified electrode. The results for determination of LD in urine samples are summarized in Table 3. The average recovery based on this method was found to be about 96 % in urine samples. The RSD of each sample was less than 1.76 %. Good recovery obtained for the spiked samples demonstrates that there is no interference from the urine content after dilution with just the buffer solution and that this is a reliable method for the direct determination of LD in urine samples. All of these results reveal that the modified electrode is effective in the determination of LD in pharmaceutical formulations and urine.

4 Conclusion

This study has demonstrated that reduced graphene oxide modified GCE can be considered as a sensitive and selective sensor in voltammetric determination of LD in the presence of AA and UA. The step-by-step CV and DPV investigations showed that the electrocatalytic activity of the modified electrode was effective in lowering the anodic overpotential for the oxidation of LD and AA by 318 and 210 mV, respectively; significantly enhancing the peak current; and successfully resolving of the overlapping voltammetric responses of LD, AA, and UA into three well-defined voltammetric peaks. The detected peak potential differences between AA and LD and between LD and UA are large enough to allow simultaneous determination of the different molecules in mixtures without significant interference. The proposed electrode showed high sensitivity and selectivity, low detection limit, good linearity, and reliable recovery data in pharmaceutical tablets and urine samples, making it appropriate for use in the electrochemical determination of LD in real samples.

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