교육학 석사 학위논문

A Selective Determination of Levodopa in the Presence of Uric Acid and Ascorbic Acid Using a Glassy Carbon Electrode Modified with Graphene

그래핀 탄소 유리 전극을 이용한
아스코르브산과 요산에서의
레보도파의 선택적 농도 결정 연구

2014년 2월

서 울 대 학 교 대 학 원
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이 논문을 교육학 석사학위 논문으로 제출함
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CONTENT

List of Schemes and Tables

List of Figures

Abstract

A Selective Determination of Levodopa in the Presence of Uric Acid and Ascorbic Acid Using a Glassy Carbon Electrode Modified with Graphene

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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 grapheme. The graphene was synthesized chemically by Hummers method and characterized by energy-filtered transmission electron microscopy (TEM). The graphene modified GCE showed excellent electro-oxidation of LD in 0.1 M PBS (pH 7.0) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The grapheme modified GCE offered significantly lower overpotential and high oxidation peak current for electro-oxidation of LD compared with bare GCE. Also, three well-resolved oxidation peaks of LD, AA and UA were obtained in their mixture solution.

Two calibration curves for LD were obtained in the range of 2 − 50 μM and 50 − 100 μM. The detection limit for LD in the lower range region was found to be 1.13 μM. The present electrode system was also successfully applied to estimate the concentration of LD in the commercially available tablets and urine samples.

Key words: Levodopa, Ascorbic acid, Uric acid, Graphene, Electrochemical sensor, Voltammetry

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1. Introduction

Parkinson's disease is a chronic neurodegenerative disease which causes progressive movement disorders when a certain part of the mid-brain dies and fails to produce enough dopamine, a neurotransmitter (chemical messenger in the nervous system) [1]. This condition 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 effectively be available for the treatment of this severe disease [2]. Levodopa (3,4-dihydroxy-1-phenylalanine) was introduced in the 1960s for the treatment of Parkinson's disease [3]. Levodopa (LD) is one of the catecholamines and a essential precursor of dopamine. This catecholamine drug, in contrast to dopamine, is able to penetrate the blood-brain barrier and is metabolized by an enzymatic reaction (dopa-descarboxilase) to dopamine, increasing concentration of dopamine in the brain [4]. However, the enzymatic metabolization of LD does also occur 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 exhibited that a powerful toxin of LD is fatal to the culture of neurons and chronic LD may be toxic in vivo, too [7]. Therefore, development of a simple, economical and precise analytical method for the determination of LD would be important for analytical applications in pharmaceutical preparations.

In order to support the evaluation of LD in pharmaceutical formulations and

biological fluid, many techniques have been developed for its determination. Several techniques for the determination of LD in pharmaceutical formulations and biological sample, including spectrophotometry [8-9], chemiluminescence [10-11], high-performance liquid chromatography [12-13] and ^{1}HNMR [14]. Nevertheless, each technique has various disadvantages associated with cost and selectivity, the use of organic solvents, complex sample preparation process and long analysis time. Compared with these methods, electrochemical techniques are more appropriate for real-time detection because of their simplicity, low-cost, possibility, good sensitivity, accuracy and rapidity for the determination of biologically important substances.

As catecholamine compounds such as LD are electroactive [15], herein, LD can also be oxidized with electrochemical technique. Unfortunately, most unmodified solid electrodes show a slow electron transfer for the electrochemical oxidation of LD with high overpotential. There are few papers on the determination of LD in pharmaceutical formulations using the modified electrodes [16-21]. The main problem of measuring of LD in vivo is the large excesses of interfering substances such as ascorbic acid (AA) and uric acid (UA). Furthermore, at most solid electrode, AA and UA are oxidized at a potential close to that of LD, resulting in a overlapping voltammetric response [22]. To resolve these problems, many chemically modified electrodes have been reported for the LD determination [23-25].

Graphene is a monolayer sheets 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 [26-27], unusual transport performance [28-29], high mechanical strength [30-31], and significantly high thermal conductivity [32-33]. The distinctive properties attracted enormous attention from fundamental research and possible applications. It has been used to fabricate a new generation of electrodes for electrochemical researches due to its great electrocatalytic activity towards some important biomolecules [34-39].

In this study, a simple and highly selective graphene modified glassy carbon electrode is prepared using graphene sheets synthesized by redox method. This electrochemical sensor was used for investigating the electrochemical behavior of LD at the surface of the graphene modified GCE. 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 biological samples.

2. Experimental Section

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 ($N_2H_4 \cdot H_2O$, 64-65 % in H_2O), uric acid (UA), ascorbic acid (AA) were obtained from Sigma-Aldrich (USA). Levodopa (LD) was obtained from Tokyo Chemical Industryco. (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). Commercialized pharmaceutical tablet was purchased from MERCKSHARP & DOHME (Australia). All other chemicals were of analytical reagent grades and used without further purification. Doubly distilled water was used throughout the experiments. 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, 3mm in diameter). Counter and reference electrodes were used by a platinum

wire and a Ag/AgCl electrode filled with 3 M KCl, respectively in this work. Transmission electron microscope (TEM) image was obtained using a LIBRA 120 plus Energy-Filtering TEM (Carl Zeiss, Germany). All ultrasonic cleaning was done using a US-2510 Ultrasonic Cleaner (Branson, USA).

2.3 Synthesis of the graphene nanosheets

Graphene Oxide (GO) was synthesized by a modified Hummers method [40]. Graphite powder (0.5 g) was pre-oxidized with potassium peroxodisulfate (0.25 g), phosphorus pentoxide (0.25 g) and 80 ℃ sulfuric acid. The reaction mixture was stirred at room temperature for 6 hours. Followed by the addition of 50 mL of water, the mixture was filtered and washed with water several times. The filtrate was dried at 40 ℃ for 24 hours. Sulfuric acid and potassium permanganate were added to pre-oxidized graphite in a ice bath. After adding 23 mL of doubly distilled water, the mixture was stirred at 90 ℃ for 30 minutes. Then another 70 mL of doubly distilled water and 1.25 mL of 30 % of H_2O_2 were added to the mixture. The powder of graphene oxide was obtained by filteration and drying in an oven at 40℃ overnight.

Graphene oxide powder was dispersed homogeneously in doubly distilled water by ultrasonication for 1 hour to obtain colloidal graphene oxide solution of 1 mg mL^{-1}. Reduction of graphene oxide was carried out by adding 7 μL of hydrazine (64-65 % in H_2O) and some amounts of ammonia

(25 wt % in H_2O) necessarily to adjust the pH to the 8.5. The solution was then kept stirring at 90℃ for 1 hour. The final dispersion was filtered, washed and dried in an oven at 40 ℃ overnight to obtain the powder of graphene.

2.4 Preparation of graphene modified GCE

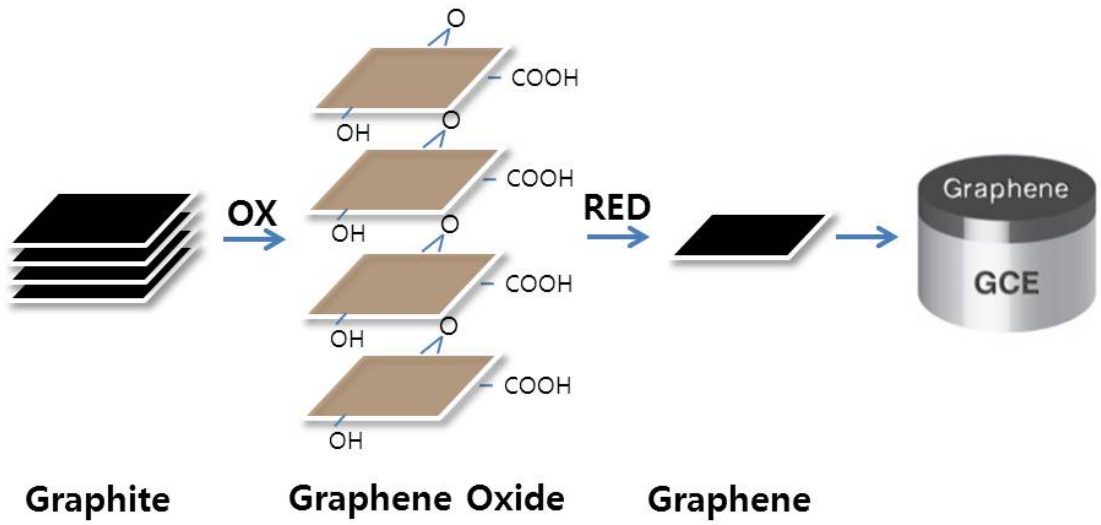
5 mg of graphene was dispersed in 10 mL of doubly distilled water with ultrasonication for 1 hour to form a black graphene suspension. Prior to the modification, the bare glassy carbon electrode was polished with 0.3 μm, 0.05 μm wet alumina powder, respectively to produce a mirror-like surface. Then it was rinsed with doubly distilled water and ultrasonicated with 50 % nitric acid, ethanol and doubly distilled water each for 5 minutes, respectively, and dried under N_2.Graphene modified electrode was prepared by coating 4 μL of graphene suspension on the surface of the electrode and dried naturally.

2.5 Preparation of LD tablets and urine samples

Five tablets (containing 100 mg of levodopa per each) were weighed and finely grinded to powder using a mortar and pestle. And then 449.6 mg was transferred into a 100 mL of flask and dissolved into 20 mL of 1% acetic acid. Next, it diluted to a final volume of 100 mL with 0.1 M PBS solution (pH 7.0). After filteration the final solution was analyzed by using the

graphene modified GCE.

The fresh urine sample was filtered and diluted 50 times using a 0.1 M PBS of pH 7.0 without any further treatment. Diluted urine sample was spiked with different amounts of LD and their concentrations were measured with DPV method.



Scheme 1. the synthetic process to make graphene at the stage of the process.

3 Results and Discussion

3.1 Characterization of graphene modified GCE

The successful synthesis of reduced graphene was confirmed by energy filtered TEM. Fig. 1 shows typical TEM image of graphene nanosheets, which reveals its rippled and crumbled few-layer planar sheet-like morphology. TEM image indicates the graphene sheets have a large surface area and length up to a few hundred nanometers, demonstrating that the exfoliation of graphene oxide down to a graphene sheet achieved under our experimental conditions.

Fig. 2 shows the cyclic voltammogram for different electrodes in 5 mM $K_3Fe(CN)_6$ in 0.1 M KCl. Compared with the bare GCE, redox peak current of $K_3Fe(CN)_6$ in 0.1 M KCl at graphene modified GCE is increased and the peak-to-peak separation (ΔE_P) is decreased. The separations (ΔE_P) of bare and modified GCE are 217 and 83mV, respectively. It was clearly demonstrated that transfer of electron on graphene modified GCE is easy and facile, compared with bare GCE.

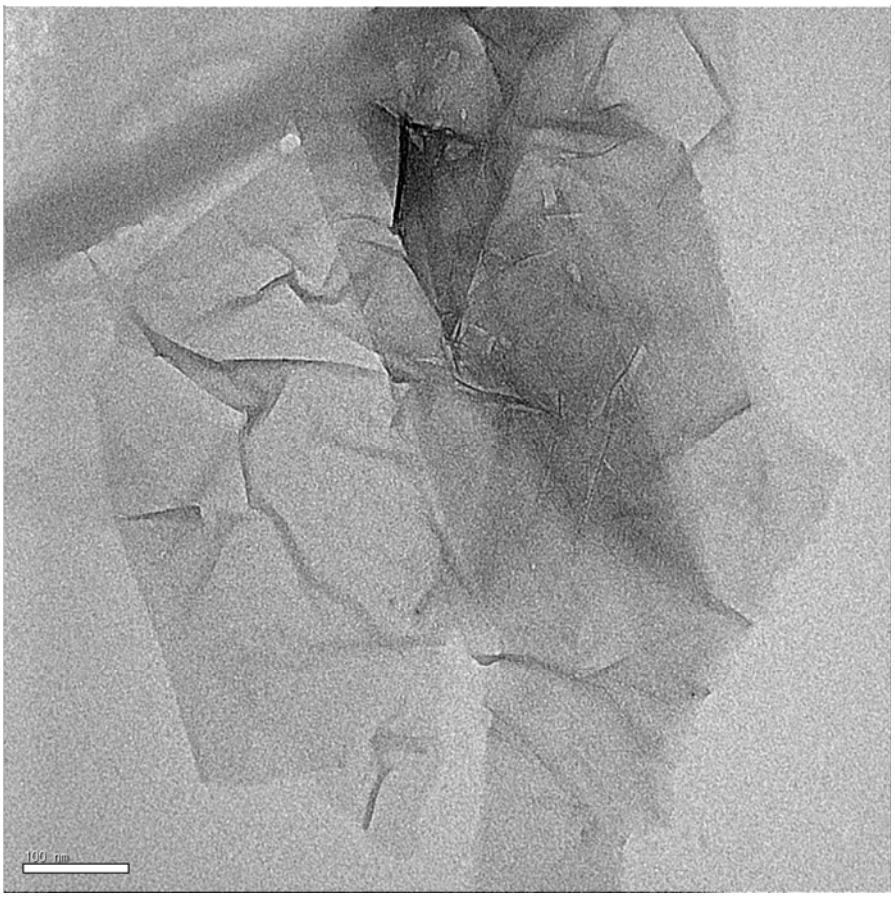


Figure 1. EF-TEM image of graphene dispersed in water.

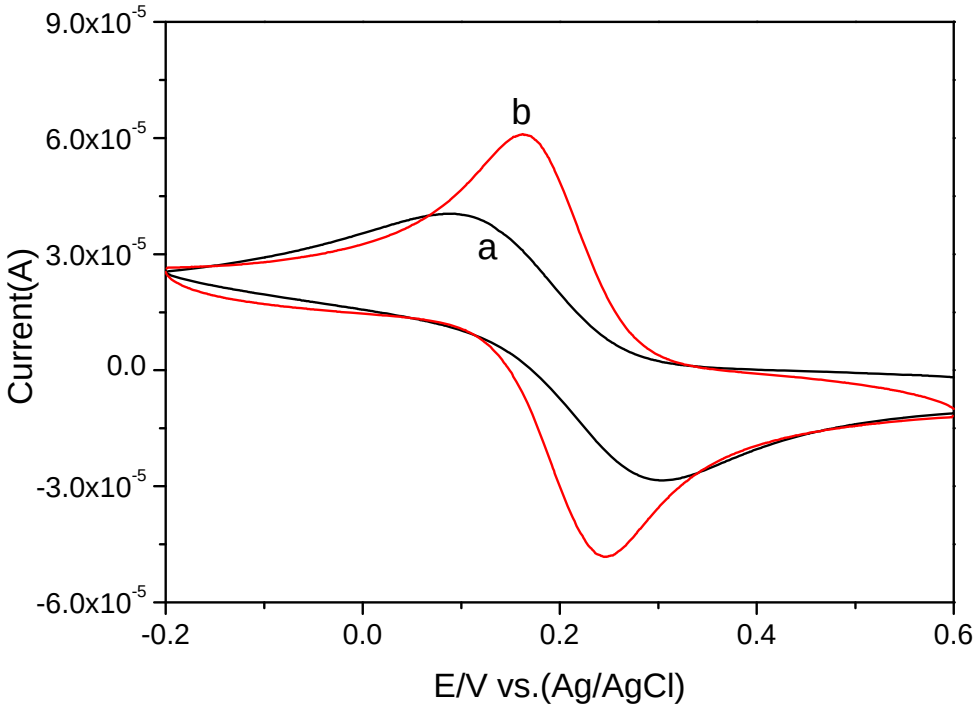


Figure 2. CVs obtained at bare GCE (a) and graphene modified GCE (b) in 0.1 M KCl containing 5 mM $K_3Fe(CN)_6$ at scan rate of 100 mV s^{-1}.

3.2 Effect of concentration of graphene suspension

To study the effect of the amount of coated graphene on GCE, the graphene modified electrode was prepared at different concentrations of graphene suspensions of from 0.1 to 1.3 mg mL^{-1}. The experiment was performed in range between 0.1 and 0.6V at a scan rate of 100 m Vs^{-1} and 3.0×10^{-3} mol L^{-1} of LD in PBS (pH 7.0). As shown in Fig. 3, the oxidation peak current increases with the concentrations of graphene suspensions from 0.1 to 0.5 mg mL^{-1}. But, as we can see in Fig. 3 (B) the oxidation peak current of LD reaches a maximum when the concentration of graphene is 0.5 mg mL^{-1}. Excessive casting of graphene increased both the peak current and the background charging current, but the increase of charging current induced to impede the increase of the cyclic voltammetric peak current. So, when the concentration of the graphene exceeds 0.5 mg mL^{-1}, the oxidation peak current of LD increase no more. In our experimental the concentration of 0.5 mg mL^{-1} was used for the determination of LD.

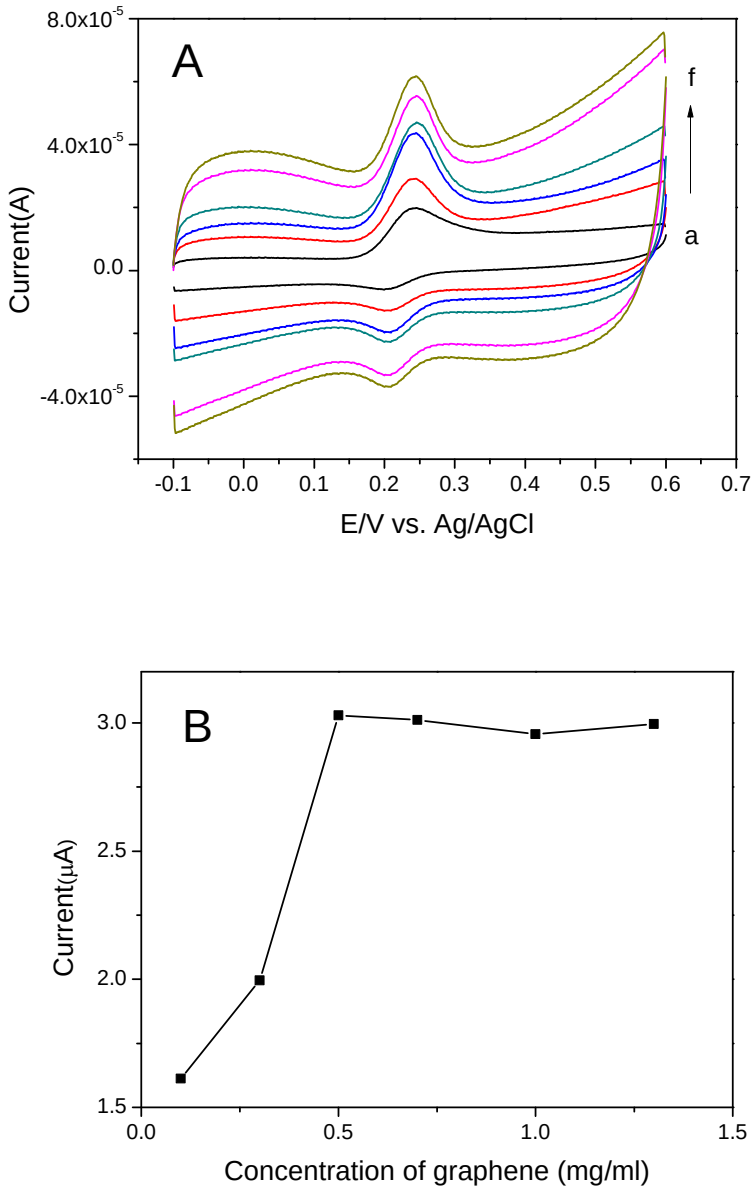


Figure 3. (A) CVs of 0.3 mM LD at the graphene modified GCE in 0.1 M PBS (pH 7.0) with different concentrations of graphene suspension from 0.1 mg mL^{-1} to 1.3 mg mL^{-1} at scan rate of 100 mV s^{-1}. The concentrations of graphene suspension : 0.1 mg mL^{-1} (a), 0.3 mg mL^{-1} (b), 0.5 mg mL^{-1} (c), 0.7 mg mL^{-1} (d), 1.0 mg mL^{-1} (e), 1.3 mg mL^{-1} (f). (B) plot of I_{Pa} vs. the concentrations of graphene suspension.

3.3 Effects of scan rate

The study of the effect of scan rate on the response of graphene modified GCE toward redox peak current of LD was carried out. Fig. 4 shows the typical cyclic voltammetric curves of LD at different scan rate, indicating that the cyclic voltammogram of LD was dependent on the scan rate. The increase in scan rate leaded to an increase in the anodic peak current and induced a shift to more positive potential value, confirmed that electrochemical reaction is not enough fast to reach the equilibrium. The oxidation peak current increased with increasing scan rate from 20 to 320 mV s^{-1} in proportion to the square root of the scan rate. (Fig. 4 (B)). The linear progress equation of I_{Pa} is I_{Pa} = -3.017 + 2.956 $\nu^{1/2}$, R^2 = 0.999. This result suggest that the electrochemical behaviors of LD on graphene modified GCE is controlled by diffusion progress.

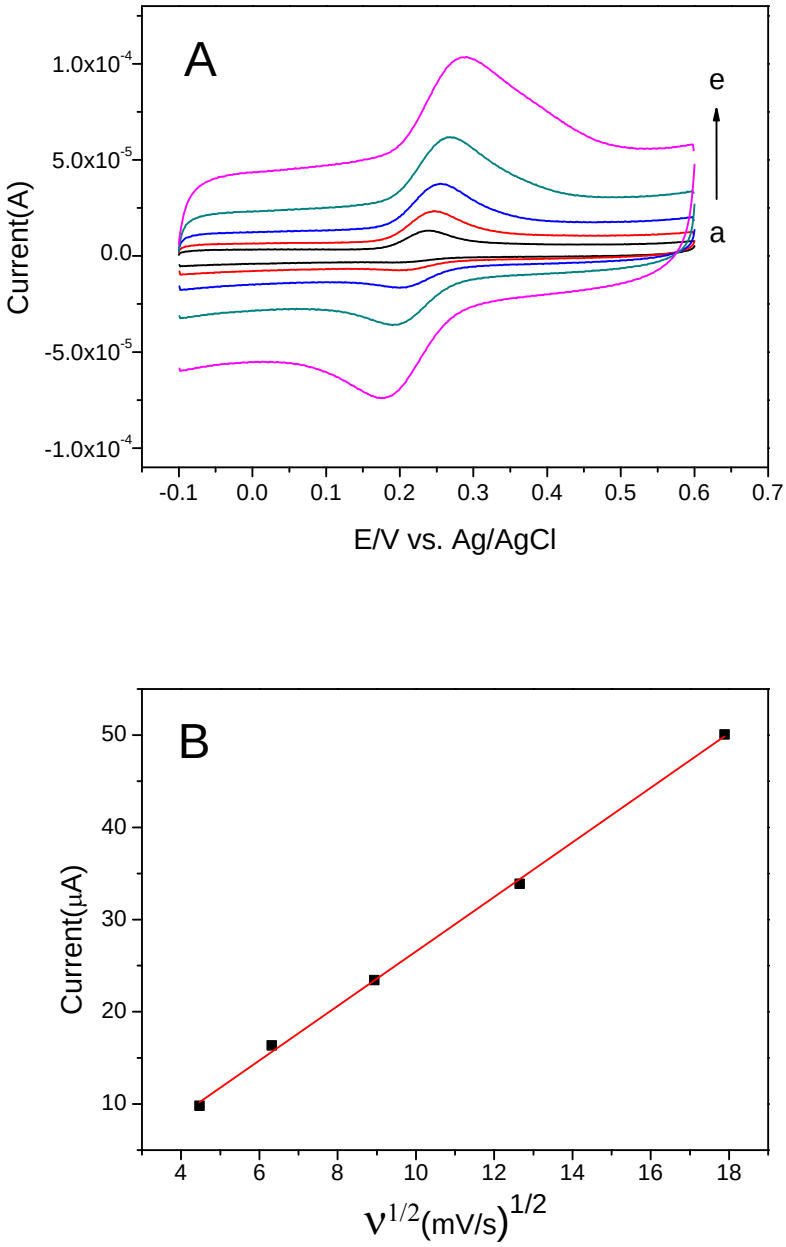


Figure 4. (A) CVs of 0.3 mM LD at the graphene modified GCE in 0.1 M PBS (pH 7.0) with different scan rate from 20 mV s^{-1}to320mVs^{-1}. Scan rates from (a) to (e) correspond to scan rates of 20, 40, 80, 160, 320, respectively (in mV s^{-1}). (B) plot of E_{Pa} vs. the square root of scan rate.

3.4 Effect of solution pH

Cyclic voltammetry was carried out to investigate the effect of solution pH on the electrochemical signal of LD at the graphene modified GCE. As shown in Fig. 5 redox peak potential of LD negatively shift with increasing pH value of solution, indicating that protons take part in the redox reaction of LD. The formal potential (E_{P}') [$E_{P}' = (E_{Pa}+E_{Pc})/2$] of the LD is linearly proportional in the pH range of 2.0 to 9.0. The linear regression of equation was $E_{P}' = -0.05465\ pH + 0.63773$, $R^2 = 0.995$. According to the Nernst equation, when the slope value is 0.59 mV pH^{-1}, the proportion of the number of electrons and protons involved in the reactions was 1:1 [41]. The slope of 55 mV pH^{-1} at the redox process of LD was close to that given by the Nernst equation, demonstrating that the number of electrons and protons took part in the redox reaction of LD is equal. In addition, oxidation peak current reach a maximum at pH 7.0 in the range of 2.0 to 9.0. In our experiment 0.1 M PBS of pH 7.0 was selected as the buffer system.

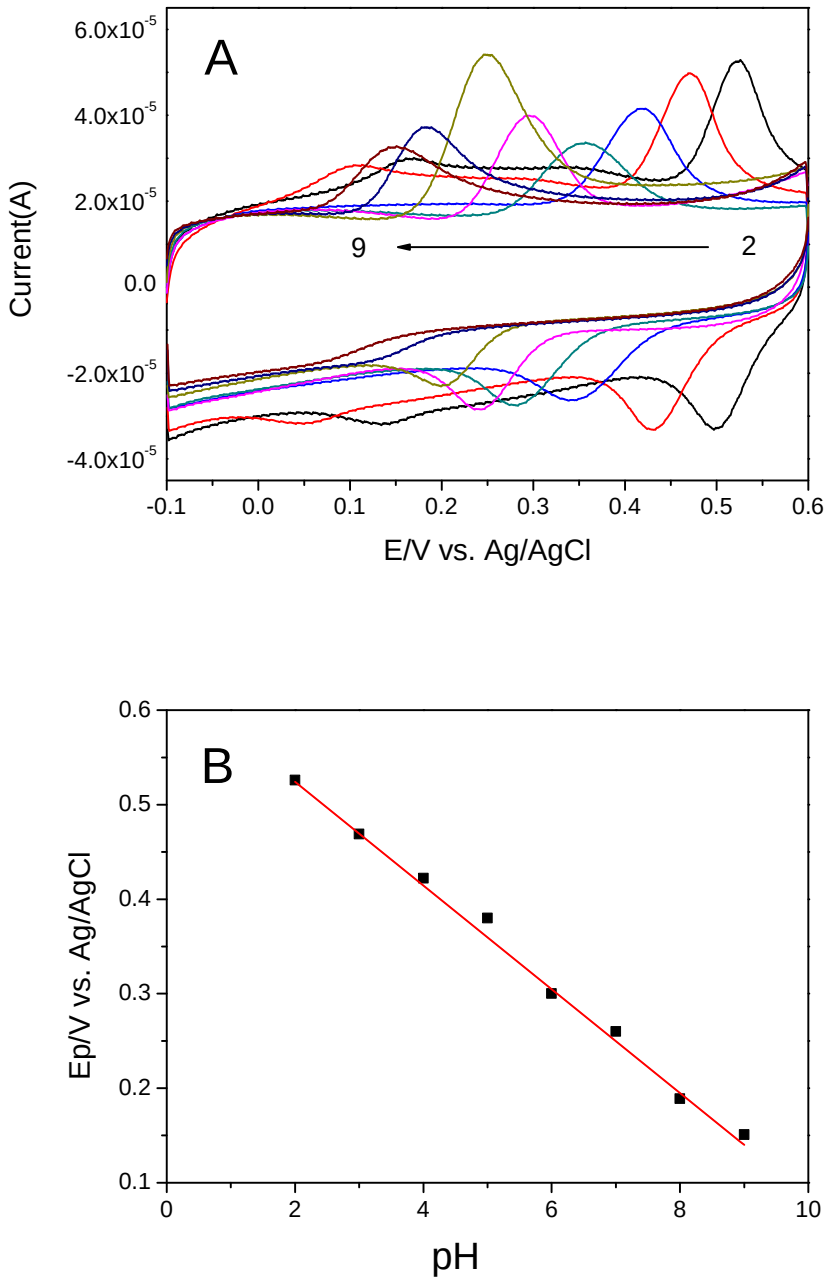


Figure 5. (A) CVs of 0.3 mM LD at the graphene modified GCE in 0.1 M PBS (pH 7.0) with different pH value from 2 to 9 at scan rate of 100 mV s^{-1}. (B) plot of E_P vs. pH.

3.5 Electrochemical behavior of LD by using graphene modified GCE

The electrochemical behavior of LD was investigated with cyclic voltammetry in 0.1 M PBS at pH 7.0. As shown in Fig. 6 a weak and broad response at the very positive potential for LD is observed on a bare GCE, revealing that the electrode process is very sluggish. A broad oxidation peak of LD is observed at ca. 0.48 V. On the other hand, using the graphene modified GCE, a well defined and very sharp anodic peak of LD is observed at 0.27 V, indicating that the graphene modified GCE can effectively decrease the oxidation potential of LD by 0.21 V. In addition, a pair of redox peaks is observed at the graphene modified GCE, with a peak-to-peak separation of about 57 mV. Furthermore, the oxidation peak current of LD at graphene modified GCE is enhanced greatly with approximately 5 times higher than that of the bare GCE. These results confirm that the graphene modified electrode improves electrochemical reactivity towards the oxidation of LD.

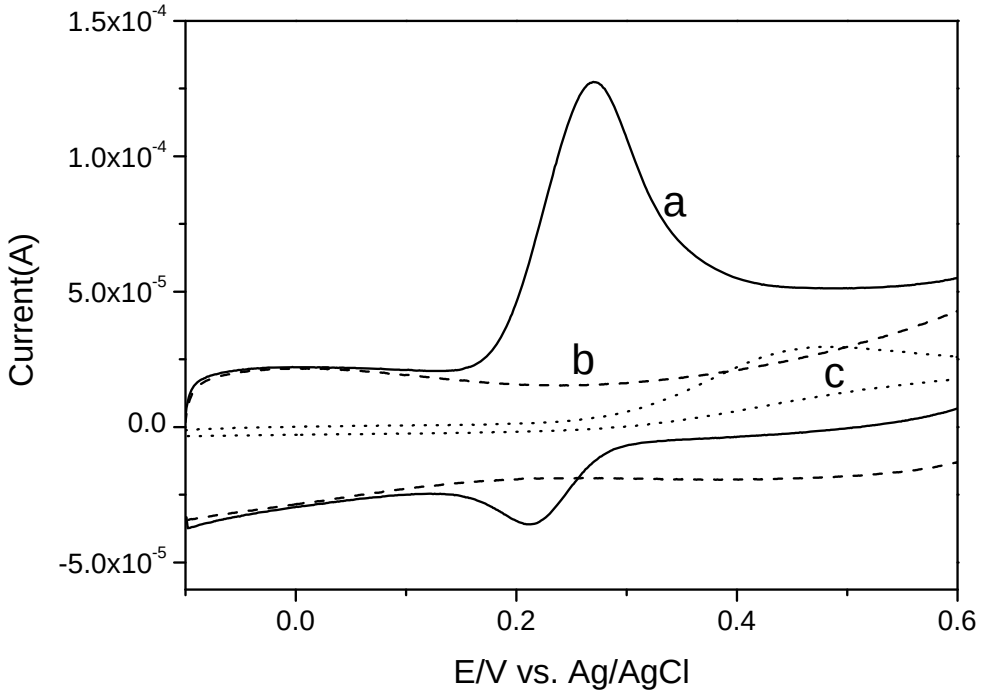


Figure 6. CVs of the graphene modified GCE in the presence (a) and absence (b) of 1mM LD and bare GCE (c) in the presence of 1 mM LD in 0.1 M PBS (pH 7.0) at scan rate of 100 mV s^{-1}.

3.6 Electrocatalytic oxidation of LD, AA and UA

In oxidative determinations of LD by electrochemical methods, AA and UA is the major hampering species. This is primarily because that AA and UA can be oxidized at almost the same potential as the LD and the concentrations of AA and UA are relatively high in biological samples [42]. Thus it is important to investigate the selective determination of LD in the presence of AA and UA. Fig. 7 shows the voltammetric responses of the bare GCE and graphene modified GCE towards LD, AA and UA. As can be seen in Fig. 7, AA, LD and UA show irreversible oxidation peaks at 0.42 V, 0.48 V and 0.463 at the bare GCE, respectively. It is not enough to obtain the selective determination for these species at the bare GCE. Whereas for the graphene modified GCE (shown in Fig. 8), the anodic oxidation peaks of AA, LD and UA appear at about 0.102 V, 0.27 V and 0.424 V, respectively. A substantial negative shift of AA, LD and UA oxidation peaks are found due to a catalytic activity of the electrode material. The difference between AA and LD peak potentials is about 168 mV, which is enough to distinguishing LD from AA. Also, at the bare electrode, the separation (ΔE_P) was not founded, but at the graphene modified electrode, the value of ΔE_P is 57 mV, indicating that the reversibility of LD at the graphene modified GCE is significantly enhanced. Fig. 8 shows that the oxidation peak currents of LD, AA and UA at the modified GCE is much improved with approximately 7, 2 and 5 times higher, respectively, compared with the bare GCE. This

may be due to the increased surface area and the catalytic activity of the graphene modified GCE. The above result demonstrates that the graphene modified GCE can not only accelerate the oxidation of LD, AA and UA, but also significantly increase the peak separations among LD, AA and UA. The enlarged separations of the anodic peak potentials, involved with the increased sensitivity, enable simultaneous determination of LD, AA and UA.

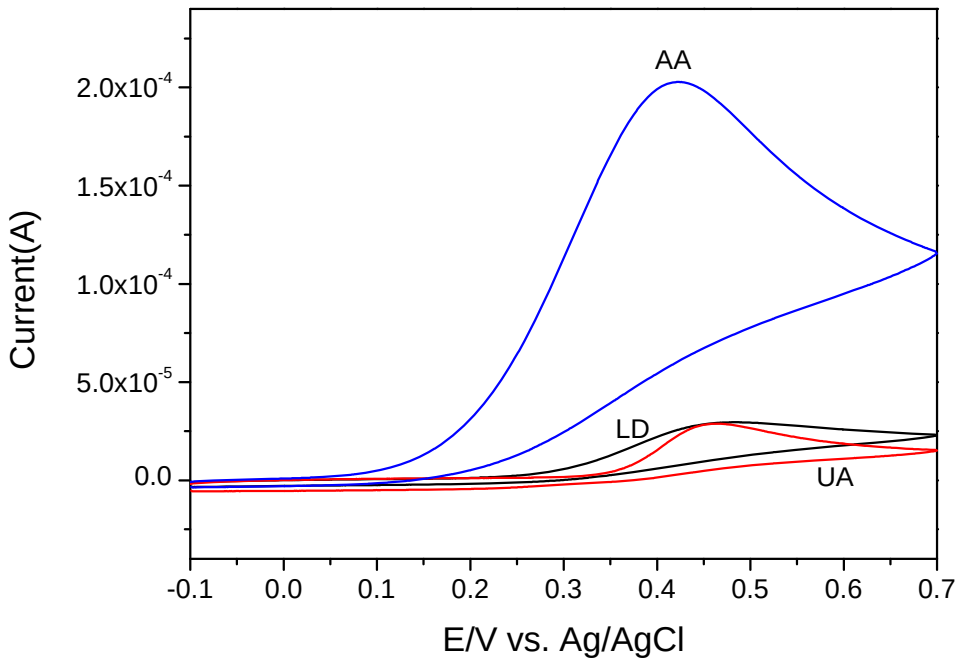


Figure 7. CVs of the bare GCE in 0.1 M PBS (pH 7.0) containing 10 mM AA, 1 mM LD and 1 mM UA at scan rate of 100 mV s^{-1}.

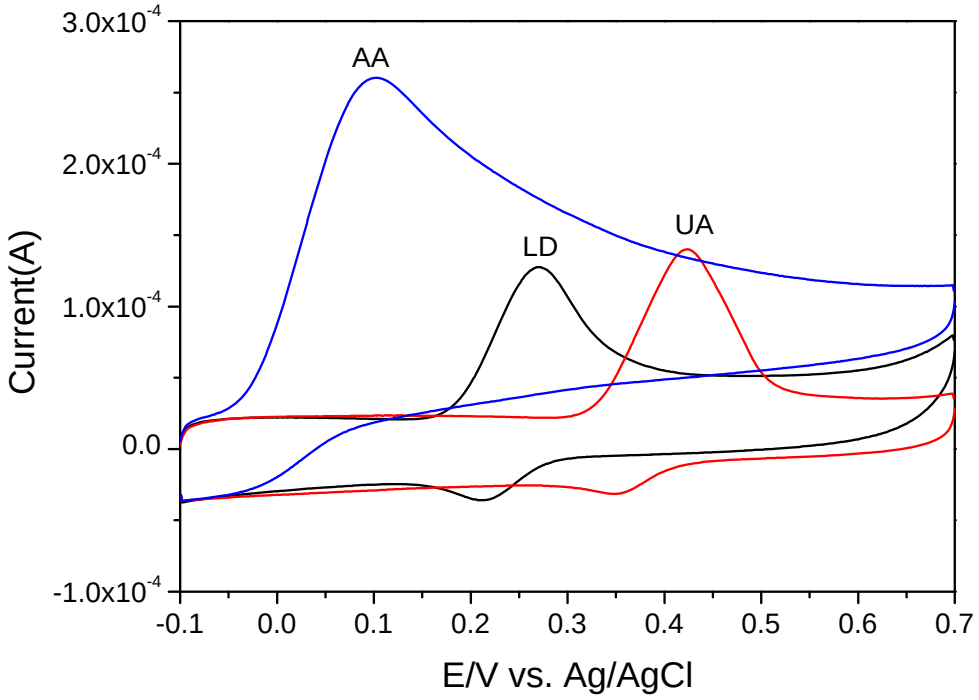


Figure 8. CVs of the graphene modified GCE in 0.1 M PBS (pH 7.0) containing 10 mM AA, 1 mM LD and 1 mM UA at scan rate of 100 mV s^{-1}.

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

Fig. 9 (A) shows cyclic voltammograms of bare GCE and graphene modified GCE in 0.1 M PBS solution (pH 7.0) containing the mixture of 0.5 mM LD, 5 mM AA and 0.3 mM UA. As shown in curve in inset of Fig. 9 (A), completely overlapped anodic peak is observed for bare GCE at around 0.438 V. In contrast, curve (a) in Fig. 9 (A) shows that modification of GCE can effectively distinguish the merged voltammetric peak into three well-resolved oxidation peaks at potentials around 0.098 V, 0.285 V and 0.423 V for AA, LD and UA, respectively. Similar peak resolution was also observed and shown in Fig. 9 (B) when differential pulse voltammetric technique was used with the electrodes in 0.1 M PBS solution (pH 7.0) containing the mixture of 500 μM AA, 20 μM LD and 20 μM UA. The voltammetric response at bare GCE in inset of Fig. 9 (B) was observed only one weak and broad peak, indicating that selectivity and sensitivity are very poor. While in curve (a) in Fig. 9 (B) the graphene modified GCE was used, the current largely increased as found with the cyclic voltammetry technique in Fig. 9 (A) and three apparent and well-defined voltammetric peaks at 0.02 V, 0.208 V and 0.336 V for AA, LD and UA, respectively were found. The observed large separations of the peak potentials allow selective determination of LD in the presence of AA and UA. This may be attributed to the outstanding electronic conductivity and high specific surface area of graphene, indicating great utility of graphene for electrochemical simultaneous

determination of LD, AA and UA.

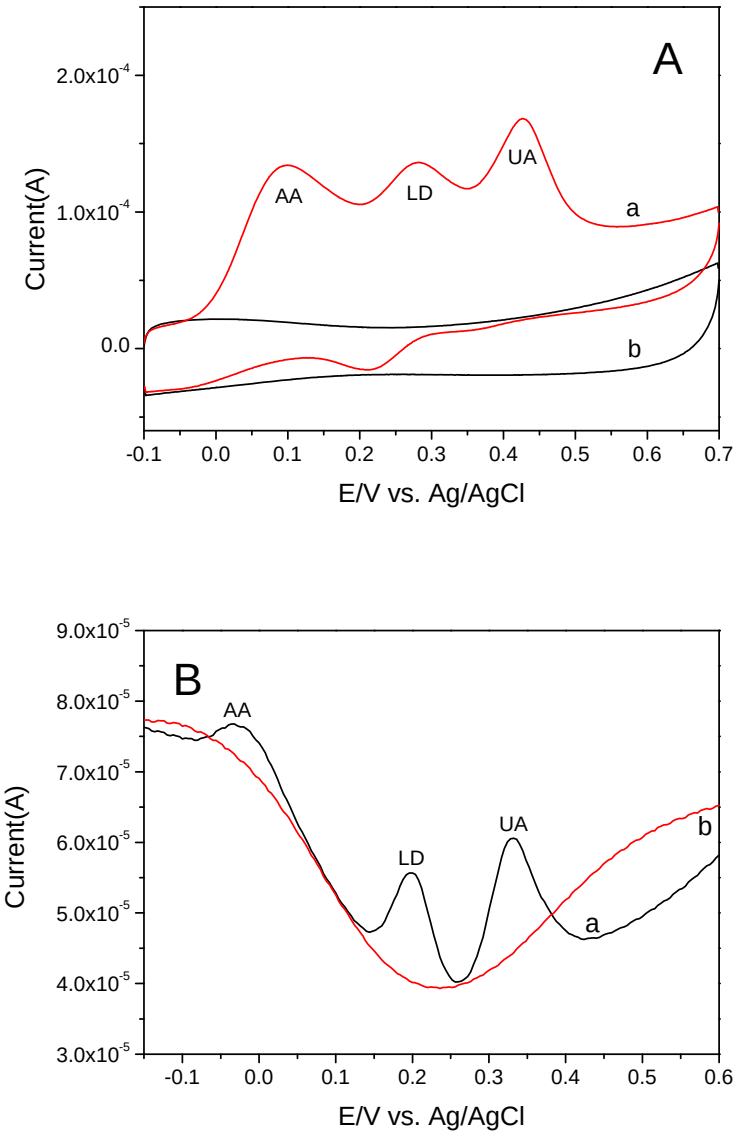


Figure 9. (A) CVs of the graphene modified GCE in the presence (a) and absence (b) of 5 mM AA, 0.5 mM LD and 0.3 mM UA in 0.1 M PBS (pH 7.0) at scan rate of 100 mV s^{-1}. Inset is CV of the bare GCE in the same mixture solution as describe before. (B) DPV of the graphene modified GCE in the presence (a) and absence (b) of 500 μM AA, 20 μM LD and 20 μM UA in 0.1 M PBS (pH 7.0). Inset is DPV of the bare GCE in the same mixture solution as describe before.

3.8 DPV determination of LD in the presence of AA and UA

The quantitative determination of LD was carried out with the DPV using the proposed graphene modified GCE. DPV is commonly used for the determination of catecholamine compounds because of its high sensitivity and resolution, which is due to small contribution of charging current to the background current [43]. In the electrochemical measurements, the concentration of LD was continuously increased with the successive addition of its standard solution, while the concentrations of the other interfering species remained constant.

Fig. 10 shows that the oxidation peak current of LD increases with increasing the amount of LD. As shown in the inset in Fig. 10, 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 μM to 50 μM in the mixture solution. The calibration equation is $I_{P,\ LD}$ (μA)=-0.22209+0.59059 C_{LD} (μM) with a correlation coefficient of R^2=0.995. The detection limit for LD in the lower range region was found to be 1.13μM.

To investigate interferences of AA and UA in the accurate determination of LD, increasing additions AA and UA are added to a solution containing 10 μ M LD. As we can see in Fig. 11, 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, the graphene modified electrode seems to be great usefulness for making voltammetric sensor for detection of LD in mixture solution.

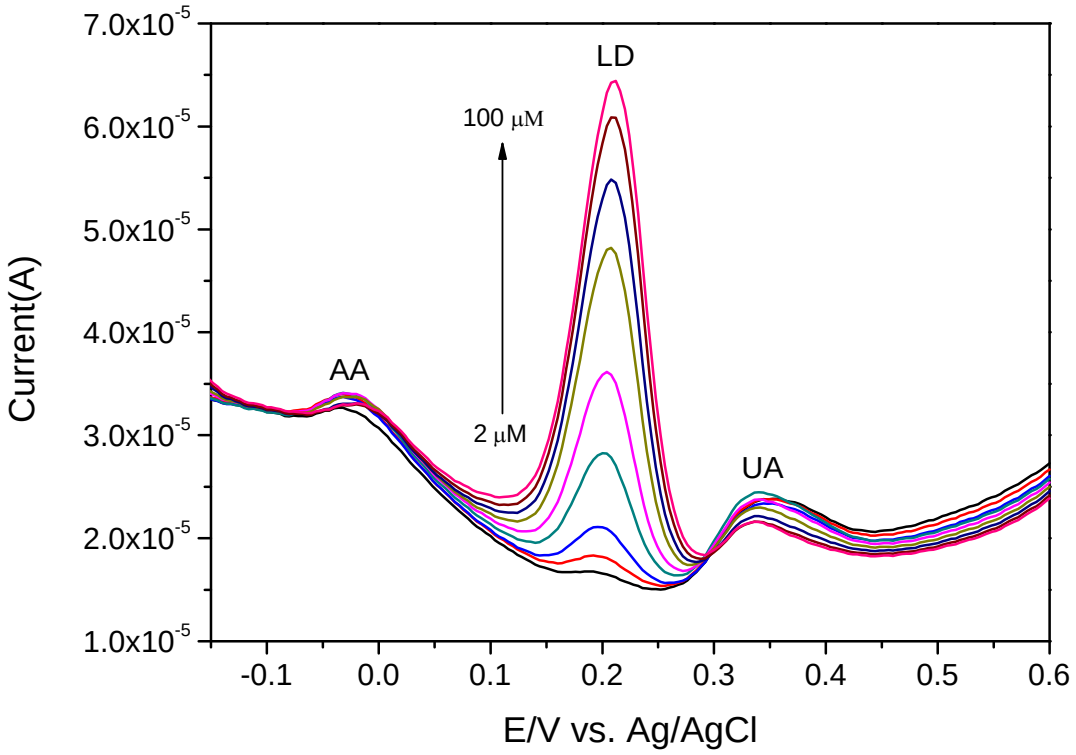


Figure 10. DPVs of the graphene modified GCE in the presence of 500 μM AA and 10 μM UA in 0.1 M PBS (pH 7.0) with different concentrations of LD.

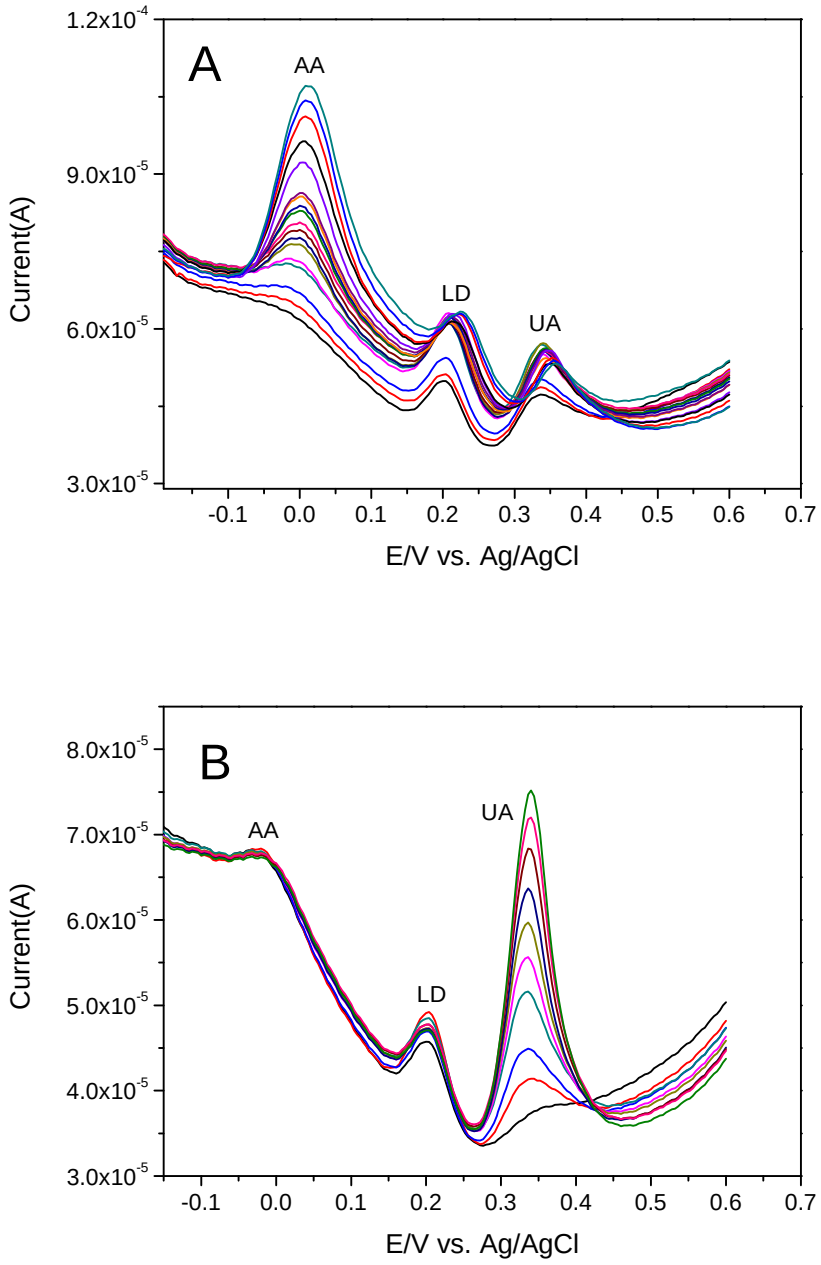


Figure 11. (A) DPVs of the graphene modified GCE in the presence of 10 μM LD and 10 μM UA in 0.1 M PBS (pH 7.0) with different concentrations of AA. (B) DPVs of the graphene modified GCE in the presence of 500 μM AA and 10 μM LD in 0.1 M PBS (pH 7.0) with different concentrations of UA.

3.9. Real sample analysis

3.9.1 Determination of LD in commercial tablets

In order to evaluate the analytical applicability of the graphene modified electrode, it was successfully applied to the direct determination of LD content of pharmaceutical tablet (SINEMET). The DPV method carried out with addition of known amounts of commercial tablet samples in phosphates buffer solution. The results are summarized in Table 1. The recovery of LD by proposed analytical method for 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 a 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 by DPV method. LD was not found from urine samples, therefore samples were spiked with different amounts of LD and were analyzed by using the graphene modified electrode. The results for determination of LD in urine samples are summarized in Table 2. The average recovery based on this method was founded to be about 96 % in urine samples. And the RSD of each sample is less than 1.76 %. Good recoveries obtained for the spiked samples demonstrate that there is no

interference from the urine content after dilution with just the buffer solution and this is a reliable method for the direct determination of LD in urine samples.

All these results reveal that the modified electrode is available for the determination of LD in pharmaceutical formulations and urine.

Table 1. The application of graphene modified GCE for 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 2. The application of graphene modified GCE for 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

4. Conclusion

For the first time, glassy carbon electrode modified with graphene used for the The electro oxidation of LD at the surface of the graphene modified GCE occurs at a potential about 210 mV more negative than that of bare GCE, demonstrating that the modified GCE exhibits an excellent electrocatalytic activity towards the oxidation of LD in phosphate buffer solution. Due to the unusual properties of graphene, the modified GCE successfully resolves the overlapping voltammetric peaks of LD, AA and UA with a potential with a 188 mV and 128 mV, respectively. So the graphene modified GCE shows high selectivity in the DPV measurement of LD, AA and UA in their mixture solutions. The modified electrode has been successfully applied to the determination of LD in real samples and the results were in agreement with those obtained by an official method. Therefore this graphene modified GCE can be used as a reliable chemical sensor of the determination of LD.

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국문요약

A Selective Determination of Levodopa in the Presence of Uric Acid and Ascorbic Acid Using a Glassy Carbon Electrode Modified with Graphene

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파킨슨 병은 만성적인 신경퇴화 질병으로 중뇌의 특정 부분에서 도파민(화학적 신경전달 물질)을 충분히 합성하지 못하여 점진적인 운동장애를 초래하는 질병이다. 이는 근육의 떨림이나 경직, 균형감의 상실과 같은 증상을 동반한다. 도파민은 뇌혈관 장벽을 통과하지 못하므로 직접 체내로 주입되었을 때 이 심각한 질병의 치료제로서 효과적으로 사용되지 못한다. 레보도파는 1960년대에 파킨슨병의 치료에 혁신을 가져왔다. 레보도파는 카테콜아민의 하나로 도파민의 중요한 전구체이다. 이것은 도파민과 달리 뇌혈관 장벽을 통과하여 효소 반응에 의해 대사됨으로써 뇌에서의 도파민의 농도를 증가시킬 수 있다. 그러나 레보도파의 대사는 중추신경계뿐만 아니라 말초신경계에서도 일어나므로 이것의 만성적인 투여는

구토, 메스꺼움, 이상 운동증과 같은 부작용을 동반한다. 또한 많은 임상 실험을 통해 레보도파의 강한 독성에 대한 연구가 보고되고 있다. 그러므로 레보도파를 검출하는 간단하고 경제적이며 정확한 분석 방법이 매우 필요하다고 볼 수 있다.

레보도파와 같은 카테콜아민은 전기화학적 활성이 있으므로 전기화학적인 방법으로 산화될 수 있다. 그러나 대부분의 전극은 레보도파의 전기화학적 산화에 있어서 전자전달 속도가 느리고 높은 과전압을 나타낸다. 또한 체액에 다량 포함된 아스코르브산이나 요산과 같은 생분자의 산화전압은 레보도파의 산화전압과 매우 비슷하여 레보도파를 혼합용액에서 선택적으로 검출하는 것이 어렵다. 따라서 수정 전극을 통한 레보도파의 선택적 검출이 필요하다.

그래핀은 탄소 원자의 sp^2 결합으로 이루어진 한 층의 막으로 이루어진 이차원 평면구조를 가지는 물질이다. 그래핀의 넓은 표면적, 뛰어난 전자전달 능력, 높은 전기전도성과 같은 우수한 성질은 전기화학적 연구를 위한 전극재료의 새로운 시대를 여는데 큰 기여를 하였다.

본 연구에서는 화학적인 산화환원 방법으로 그래핀을 합성하고 이를 이용하여 그래핀 탄소 유리 전극을 제조하였다. 이러한 전기화학적 바이오센서는 순환 전압전류법과 시차펄스 전압전류법을 이용하여 레보도파의 전기화학적 거동을 연구하는데 사용되었고 그 결과 탄소 유리 전극에 비해 레보도파의 전기화학적 산화 신호가 크고 분명하게 나타났다. 또한 그래핀 탄소 유리 전극은 높은 농도의 아스코르브산과 요산이 함께 혼합되어 있는 용액에서 레보도파를 선택적으로 검출할 뿐만 아니라 정량적인 농도 결정이 가능함을 확인하였다. 최적 조건 하에서, 산화 피크의 전류값은 레보도파 농도 2.0×10^{-6} M ~ 1.0×10^{-4} M의 범위 내에서 농도에 선형적으로 비례하였으며, 검출 한계는 1.1×10^{-6} M 이었다. 마지막으로,

본 연구에서 개발한 전극은 실제 소변내의 레보도파의 농도를 검출하는 데에도 성공적으로 적용할 수 있었다. 또한, 실제 판매되고 있는 레보도파의 농도 검출을 확인함으로써 이 그래핀 탄소 유리 전극은 실제 샘플에도 적용할 수 있을 것으로 기대된다.

핵심 되는 말: 레보도파, 아스코르브산, 요산, 그래핀, 전압전류법, 전기화학 센서

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