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Full Paper

Electro-analysis of Orphenadrine Hydrochloride by Graphene Modified Glassy Carbon Electrode and Its Oxidation Mechanism

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Abstract- Electro-analysis of orphenadrine (ORD) by graphene modified glassy carbon electrode (GPN/GCE) was studied using cyclic voltammetric and linear sweep voltammetric (LSV) techniques. The variation of the current with pH, concentration and scan rate was investigated to optimize the experimental condition for determination of ORD. The electrochemical behavior of the ORD at GPN/GCE was a diffusion-controlled process. A probable electro oxidation mechanism was proposed. Under the optimal conditions, the anodic peak current was linearly proportional to the concentration of ORD in the range from 0.03-0.37 μg/mL with a limit of detection 8.6×10^{-4} μg/mL for LSV. This method was applied for quantitative determination of ORD levels in urine as real samples. Further interference study was also carried with some common interfering substances.

Keywords- Orphenadrine; Voltammetry; Graphene; Glassy carbon electrode; Oxidation

1. INTRODUCTION

Drug determination is one of the important rules for drug quality control. Therefore, the development of simple, sensitive, rapid and reliable method for the determination of drug is of great importance. Orphenadrine hydrochloride (N, N, dimethyl-2(o-methyl-alpha-phenylbenzyloxy) ethylamine hydrochloride) (ORD) is a widely used anti-cholinergic drug [1-

6] of the ethanolamine antihistamine class with prominent central nervous system (CNS) and peripheral actions used to treat painful muscle spasm as well as some aspects of parkinson's disease, a neurological disorder. It is also widely used in psychiatric medicine for the property correlations, charge transfer mechanism, etc, based on density functional theory (DFT) calculations [7]. Cardiovascular non-sustained ventricular tachycardia has been attributed to orphenadrine [8]. The over dosage of orphenadrine has been stated to be relatively toxic [9]. Although some patients have survived doses grossly in excess of the usual maximum of 400 mg/day, the therapeutic margin can vary. The clinical picture of intoxication tends to be characterized by coma with seizures, apnea, disturbances of cardiacrhythm and shock; physostigmine should only be used cautiously and preferably after the most severe toxic phase has been overcome [10]. The mechanism of action of the antiparkinson agent, ORD have shown that the compound raises the level of bound 5-hydroxytryptamine (5HT) in the rat brain .

Graphene (GPN) is a two-dimensional (2-D) sheet of carbon atoms in a hexagonal configuration with atoms bonded by sp^2 bonds. These bonds and this electron configuration are the reasons for the extraordinary properties of graphene, which include a very large surface area at 2630 m^2/g, it is double that of single-walled carbon nanotubes (SWCNTs), a tunable band gap, room-temperature Hall effect, high mechanical strength (200 times greater than steel), and high elasticity and thermal conductivity [11]. GPN is an ideal material for electrochemistry [12-15]. It is used as a conductive support for the deposition of electro catalytic nanoparticles [16]. It is expected that a stable GPN-based strain sensor will be used in many fields in the future. One of the potential applications is in the touch screen, which utilizes its transparency and good electrical and mechanical properties .

To the best of our knowledge, there are no reports based on graphene modified electrode for the determination of ORD. Several methods have been reported for the determination of ORD such as spectrophotometry [17], high-performance liquid chromatography [18,19], capillary electrophoresis methods [20], the voltammetric technique for determination of ORD at gold electrode [21]. However, these methods suffer from some disadvantages such as high cost, long analysis time, sample pre-treatment, low sensitivity and selectivity, which make them unsuitable for routine analysis. Hence, we have undertaken the development of electrochemical method for the determination of ORD at graphene modified electrode in this paper.

2. EXPERIMENTAL SECTION

2.1. Reagents and Chemicals

The powdered form of ORD was obtained from Sigma Aldrich and used without further purification. A stock solution (1.0 mM) of ORD was prepared in double distilled water. The phosphate buffers from pH 3.0-11.2 were prepared in double distilled water as described by

Christian and Purdy [22]. All chemicals used were of reagent grade and double distilled water was used throughout the work. A solution of ORD was prepared by dissolving an appropriate amount of sample in double distilled water. The required concentration of ORD was used from its aqueous solution.

2.2. Instrumentation

Electrochemical measurements were performed on a CHI 630D Electrochemical Analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and GPN modified with GCE (GPN/GCE) as a working electrode. All the potentials are given against the Ag/AgCl (3.0 M KCl). All experiments were carried out at an ambient temperature of 25°C ± 0.1°C. The pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India).

2.3. Synthesis of Graphene

The graphene was prepared according to the method reported in the literature [23]. Natural flake-graphite is reacted with the concentrated sulphuric and nitric acids with potassium chlorate for 96 hours. After the graphite was oxidized , the mixture was added to the excess water, washed with 5% solution of HCl, and repeatedly washed with water until the pH of the solution was 7.0 (neutral solution). Then through extremely rapid heating and successful splitting of graphite oxide, wrinkled graphene sheets functionalized with hydroxyl and carboxylic groups were obtained.

2.4. Characterization of Graphene

Graphene dispersed in ethanol was characterized with TEM. Fig. 1(A). (a) shows the image of the wrinkled graphene sheet with no aggregation, indicating that the functionalized graphene sheets stable at room temperature for about 3 weeks. In Fig. 1(A). (b) material yielding an electron diffraction of nanocomposite material yielding a double six-spot-ring pattern, which confirms the benzene-ring pattern of the graphene sheet [24]. Fig. 1(B). (a) and (b) shows the SEM image of graphene film on the GCE, revealing the typical crumpled and wrinkled graphene sheet structure on the rough surface of the film which has been already reported [25].

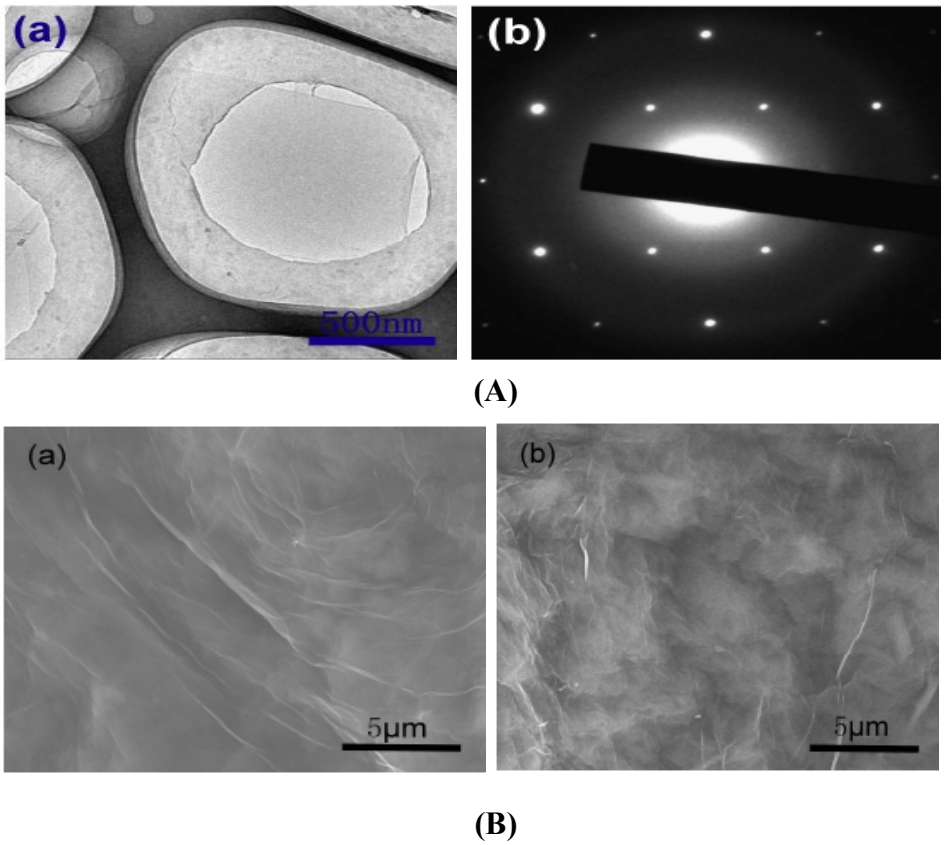

Fig. 1. A) (a) Bright-field TEM images of grapheme (b) Electron diffraction patterns for the layers of graphene; B) (a) SEM images of the surface of graphite powder (b) SEM images of the surface of graphene powder

2.5. Preparation of Bare and Graphene Modified Electrode

The bare GCE was polished with 0.5 mm alpha alumina powder before it was used, rinsed ultrasonically with 1:1 HNO_3, ethanol and double distilled water, respectively, and dried at room temperature. Graphene was dispersed in methanol (1 mg/ml) with ultrasonication for 30 minutes. Five micro liters of suspension was cast on to the surface of GCE and dried in air. Prior to use, the modified electrode was carefully rinsed with water to remove the loosely attached graphene and dried in an air stream.

By varying the scan rate, the area of the electrode was calculated using 1.0 mM $K_3[Fe(CN)_6]$ as a probe. For a reversible process, the Randles-Sevcik formula has been used [26]:

$$i_{pa} = (2.69x10^5)n^{3/2}AD_o^{1/2}C_o\,\upsilon^{1/2} \quad (1)$$

where, i_{pa} refers to the anodic peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_0 is diffusion coefficient, υ is the scan rate and C_0 is the concentration of $K_3[Fe(CN)_6]$. For 1.0 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl electrolyte, n = 1, D_0 =

7.6×10^{-6} cm^2s^{-1} [27], then from the slope of the plot of i_{pa} versus $\upsilon^{1/2}$, the surface area of electrodes was calculated and found to be 0.046 cm^2 for GCE and 0.295 cm^2 for GPN/GCE.

2.6. Voltammetric Measurement Procedures

The GPN/GCE in the three electrode system was immersed in 0.2 M phosphate buffer (pH 7.0) containing known amount of ORD. The linear sweep voltammogram (LSV) were recorded 0.4 to 1.2 V after open-circuit accumulation for 180 s with stirring. The oxidation peak current of ORD was measured, the parameter of LSV were, pulse with of 0.2 s pulse increment of 4mV, pulse period of 0.5 s pulse amplitude of 50mV and scan rate of 0.05 mV s^{-1} .

3. RESULTS AND DISCUSSION

3.1. Electrochemical Behaviour of ORD at GPN/GCE

ORD being readily oxidizable one, its voltammogram was recorded in the potential range from 0.4 to 1.2 V in the supporting electrolyte 0.2 M phosphate buffer of pH 3.0. Fig. 2 curve (iii) shows a CV profile for 1.0 mM ORD at GCE with an anodic peak. As from Fig. 2 curve (i) the introduction of graphene results cyclic voltammograms with anodic peaks of good intensity and sharpness, the anodic peaks A and B were obtained at 0.788 and 0.940 V respectively, both are irreversible. Since, peak A was more intense than peak B, peak A was considered for further experimental determination of ORD. Fig. 2 curve (ii) shows a CV profile for blank supporting electrolyte at GPN/GCE with no redox peaks. The significantly enhanced voltammetric response of ORD on GPN/GCE and the lower oxidation potential could be reasonably ascribed to the large specific surface area and electro catalytic activity of GPN film.

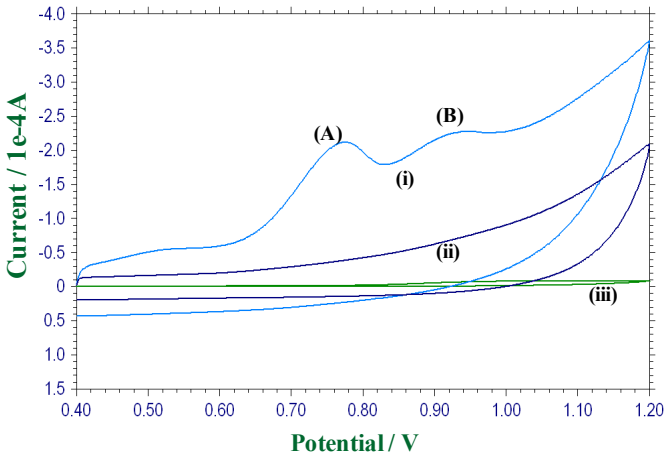

Fig. 2. Cyclic voltammograms obtained in pH 7.0, 0.2 M phosphate buffer at scan rate of 50 mVs^{-1} for (i) GPN/GCE with ORD, (ii) GPN/GCE without ORD, and (iii) GCE with ORD

For further investigating the redox properties of ORD at the GPN/GCE, successive cyclic scan was performed in a 1.0 mM ORD solution. It could be seen that the anodic peak current decreased obviously in the second scan compared with that of the first one, and gradually reduced with successive cyclic sweep. The reason may be that the oxidation product of ORD, adhered to the electrode surface and hindered the access of ORD. Accordingly, the first cyclic sweep was adopted.

3.2. Influence of pH

The effect of pH of the solution on the voltammetric response of ORD (1.0 mM) was investigated in pH range from 3.0 to 10.4. However for pH 3.0 - 4.2, the peaks were not sharp. Hence, the voltammetric analysis of ORD was restricted in the pH range from 5.0 to 10.4 as shown in Fig. 3(A). As increasing the pH, the peak potential of ORD shifted negatively from 0.96 to 0.70 V as shown in Fig. 3(B).

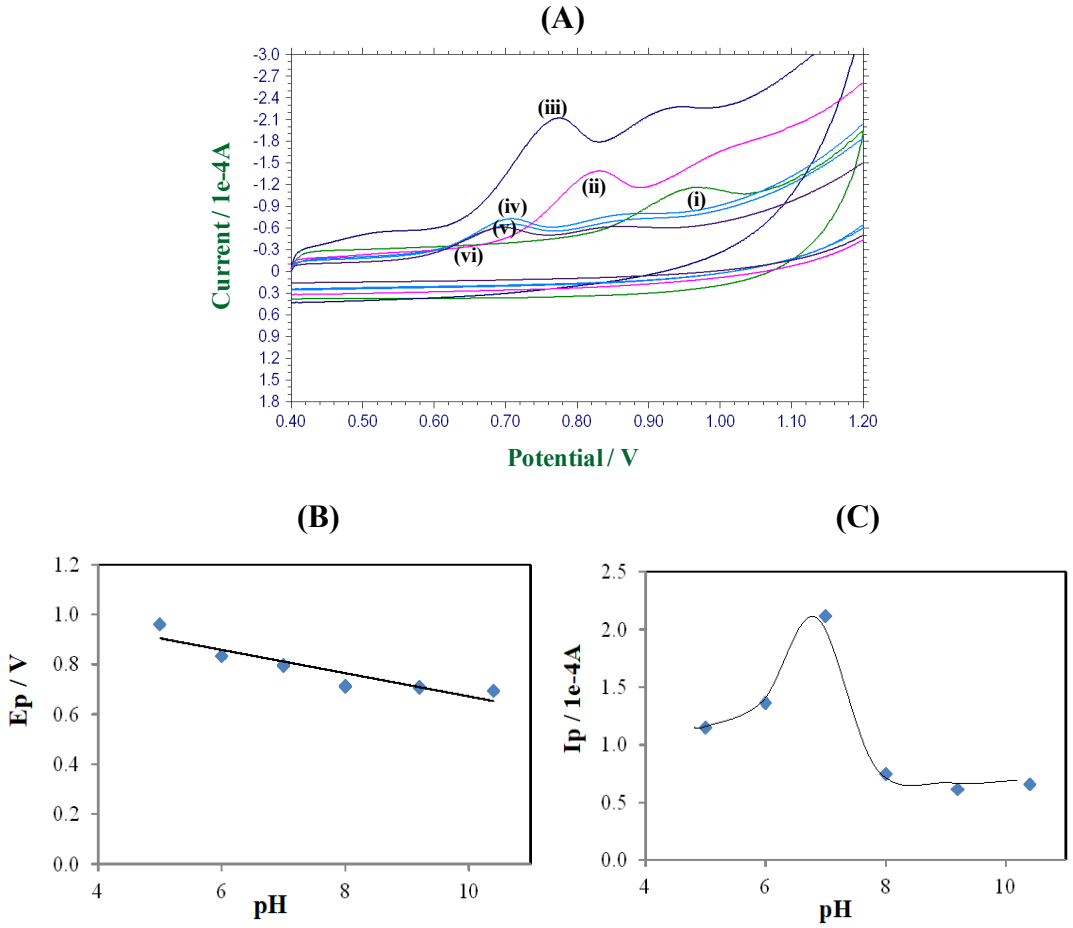

Fig. 3. A) Cyclic voltammograms obtained for 1.0 mM ORD at GPN/GCE with potential scan rate 50 mVs^{-1} in phosphate buffer solution of (i) pH 5.0, (ii) pH 6.0, (iii) pH 7.0, (iv) pH 8.0, (v) pH 9.2 and (vi) pH 10.4; B) Variation of peak potential with pH for 1.0 mM ORD; C) Variation of peak current with pH for 1.0 mM ORD

The shift in Ep with pH refers to a proton transfer in the electrochemical oxidation of ORD. It has also been concluded that the equal numbers of electrons and protons are involved in the oxidation process of ORD [28], which was confirmed by slope value of the plot of Ep versus pH. The variation of peak current with pH is shown in Fig. 3(C). From the experimental results, it is observed that highest peak current and better shape of the voltammogram was observed at pH 7.0, suggesting this pH is optimal pH value.

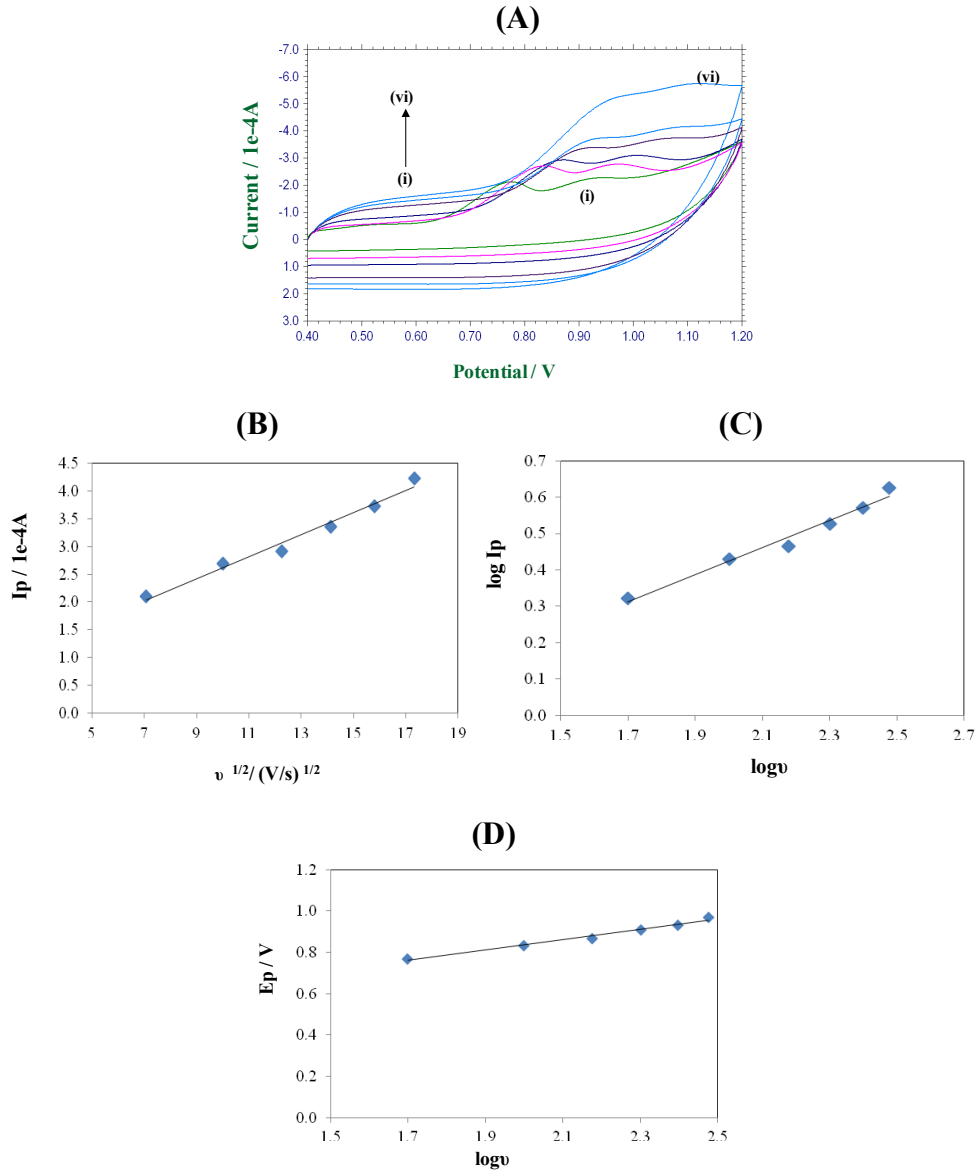

Fig. 4. A). Cyclic voltammograms obtained for 1.0 mM ORD at GPN/GCE in phosphate buffer solution of pH 7.0 at scan rates of (i) 50, (ii) 100, (iii) 150, (iv) 200, (v) 250, and (vi) 300 mVs^{-1}; B) Linear relationship between the peak currents and the square root of scan rate; C) Linear relationship between the logarithmic peak current and the logarithmic scan rate; D) Variation of peak potential with log **υ** for 1.0 mM ORD

3.3. Effect of Scan Rate

The influence of scan rate plays an important role in voltammetric oxidation reactions. So, it is important to evaluate the dependence of peak current and peak potential on the scan rate and whether the electrode reaction was adsorption or diffusion controlled process. Fig. 4(A) was the superimposed voltammograms of varying scan rates from 50 to 300 mV s^{-1}. The anodic peak current was linearly proportional to the square root of the scan rate.

The linear regression equation was expressed as Ip = 0.198 $\upsilon^{1/2}$ + 0.638 (R^2 = 0.977) as shown in Fig. 4(B). When we fitted the relationship between log Ip and log ν, a better linear regression equation was obtained: log Ip = 0.372 logν-0.319 (R^2 = 0.975) (Fig. 4(C)). The slope value was found to be 0.372 which is close to the theoretically expected value of 0.5 for a diffusion controlled process.

Moreover, with the scan rate (υ) increasing, the oxidation peak potential E_p at proposed electrode was positively shifted and the relationship between the peak potential and the logarithm scan rate was shown in Fig. 4(D). As for an irreversible electrode process, according to Laviron [29], Ep is defined by the following equation (2):

$$E_p = E^{0`} + \left(\frac{2.303RT}{nF}\right)\log\left(\frac{RTk^0}{nF}\right) + \left(\frac{2.303RT}{\alpha_n F}\right)\log \nu \qquad (2)$$

where $E^{o'}$ is the formal standard potential, k^0 is the standard heterogeneous rate constant of the reaction, α is the charge transfer coefficient, n is the number of the electron transferred in oxidation of ORD, and ν, R, F and T represent their usual meaning. Thus, the value of 'αn' can be easily calculated from the slope of Ep vs log ν plot. In this system, the slope was found to be 0.249, taking T = 298 K, R = 8.314 JK^{-1} mol^{-1} and F = 96,480 C mol^{-1}, and 'αn' was found to be 0.23. Bard and Faulkner formula [30] (equation 3) was used to evaluate the value of α.

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \text{ mV} \qquad (3)$$

The value of α was found to be 0.33. So the number of electrons (n) transferred in the electro-oxidation of ORD was calculated to be 0.69 ≈ 1.

The value of k^0 can be determined from the intercept of the above plot if the value of $E^{o'}$ is known. The value of '$E^{o'}$' in Eq. (2) is obtained from the intercept of Ep vs ν curve by extrapolating to the vertical axis [31] at $\nu = 0$ and '$E^{o'}$' was obtained to be 0.742V, the 'k^0' was calculated to be 5.05×10^{-3} s^{-1}.

3.4. Calibration curve of Orphenadrine Determination

In order to construct a broader range calibration curve for the quantitative determination of ORD at GPN/GCE, linear sweep voltammetry (LSV) was employed, with the following parameters: sweep rate: 20 mVs^{-1}, pulse amplitude: 50 mV, pulse width: 60 ms and pulse period: 500 ms. Under optimized conditions, the oxidation peak currents were found to be proportional to the concentration of ORD over the range of 0.03-0.37 µg/mL for LSV in phosphate buffer of pH 7.0. The corresponding results are shown in Fig. 5(A) and (B), the corresponding regression equation 4 is:

$$Ip(10^{-4}A) = 0.125[ORD](10^{-7}\ M) + 0.598\ ;\ R^2 = 0.972 \qquad (4)$$

The values of limit of detection and limit of quantification were calculated using the following equations 5:

$$LOD = 3s/m;\ LOQ = 10\ s/m \qquad (5)$$

where 's' is the standard deviation of the intercept of the calibration plot (five runs) and m is the slope of the calibration curve. The values of LOD and LOQ were observed to be 8.6×10^{-4} µg/mL and 27.3×10^{-4} µg/mL respectively. Lower values of LOD and LOQ indicated the sensitivity of the proposed method. Comparison of earlier methods with present method showed that, the present method is better than other reported methods for the determination of ORD (Table 1).

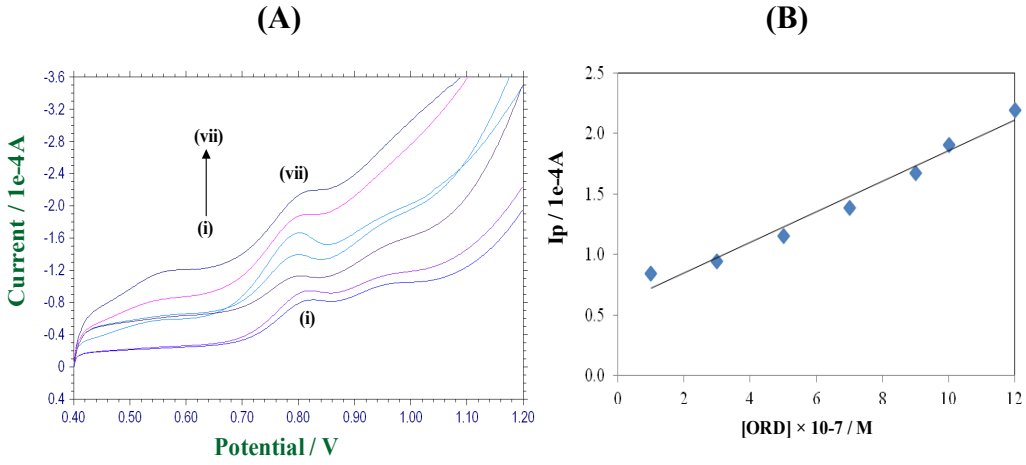

Fig. 5. A). Linear sweep voltammograms of ORD at different concentrations of (i) 1.0×10^{-7}, (ii) 3.0×10^{-7}, (iii) 5.0×10^{-7}, (iv) 7.0×10^{-7}, (v) 9.0×10^{-7}, (vi) 1.0×10^{-6}, and (vii) 1.2×10^{-6} M; (B) Plot of the peak current against concentration of ORD

3.5. Stability and Reproducibility

In order to study the stability and reproducibility of the electrode, a 1.0 µM ORD solution were measured with the same electrode for every several hours within a day, the RSD of the

peak current was 0.75% (number of measurements = 5). As to the within a day reproducibility, it was similar to that of between a day if the temperature was kept almost unchanged which could be attributed to the excellent stability and reproducibility.

Table 1. Comparison of some methods for the determination of ORD with the proposed method

Sensors Used	Linearity range (µg/mL)	LOD (µg/mL)	Reference
1) Spectroscopic	1-12	9.8	[17]
2) HPLC	3-1000	10	[18]
3) Capillary electrophoresis	8.7-5.0	0.20	[20]
4) GPN/GCE	0.03-0.37	8.6×10^{-4}	Present work

3.6. Interference Study

The interference effects were investigated by detecting the response of GPN/GCE to a 1.0×10^{-6} M ORD in the presence of different interferents. The selected interferents were some species that possibly exist in biological samples were evaluated under the optimum experimental conditions. Linear pulse voltammetric experiments were carried out for 1.0×10^{-6} M ORD in the presence of 1.0 mM of each of the interferents. The experimental results (Table 2) showed that thousand-fold excess of citric acid, glucose, gum acacia, oxalic acid, starch and sucrose did not interfere with the voltammetric signal of ORD. Therefore, the proposed method can be used as a selective method.

Table 2. Influence of potential interferents on the voltammetric response of 1.0 µM ORD

Interferents	Concentration (mM)	Signal Change (%)
Citric acid	1.0	-4.32
D-Glucose	1.0	-1.32
Gum acacia	1.0	-0.17
Oxalic acid	1.0	0.47
starch	1.0	-0.15
Sucrose	1.0	-0.19

3.7. Analytical Applications

The applicability of the GPN/GCE was investigated by the recovery test of spiked ORD in urine samples. The urine samples were diluted 100 times with the phosphate buffer solution

(pH 7.0) before analysis without further pre-treatment and determined by LSV technique. The results are summarized in Table 3. The recoveries of the samples are between 101.0 and 101.5%. The calibration graph was used for the determination of spiked ORD in urine samples. With these satisfying results, the sensing platform based on GPN/GCE can be used for the determination of ORD in real samples.

Table 3. Determination of ORD in urine samples

Urine	Spiked (µM)	Detected[a] (µM)	Bias (%)	Recovery (%)	SD±RSD (%)
Sample 1	1	1.01	-1.0	101.1	0.0026±0.03
Sample 2	2	2.02	-1.05	101.0	0.0020±0.10
Sample 3	3	3.04	-1.59	101.5	0.0028±0.09
Sample 4	4	4.04	-1.12	101.1	0.0031±0.07

(a) Average of five determinations

4. CONCLUSION

In present work, it was demonstrated that graphene modified glassy carbon electrode was used as an electrochemical sensor for determination of ORD. LSV technique has been employed for the determination of ORD in biological samples. Proposed procedure has also been successfully used for urine samples, with good recoveries obtained at the levels tested. The method is rapid, sensitive. Furthermore, the present method could possibly be adopted for studies of clinical and quality control laboratories.

Conflict Of Interest

The authors state that they have no conflicts of interests.

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