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Research Article

Electrochemical Exfoliated Graphene Oxide Nanosheets Modified Graphite Electrode for Clozapine Sensing

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ABSTRACT

Graphene oxide nanosheets (GONs) are ideally suited for implementation in electrochemical applications due to their large electrical conductivity, large surface area and low production costs. In this research, electrochemical exfoliated graphene oxide-modified graphite electrode was developed for voltammetric determination of clozapine (CLZ). The dependence of oxidation peak current on the pH of the solution, scan rate and concentration of analyte were studied to optimize the experimental conditions for electrochemical determination of CLZ. The experimental results suggested that the modified electrode promoted electron transfer reaction for the oxidation of CLZ. The modified electrode exhibited high effective surface area, more reactive sites and excellent electrocatalytic activity toward the oxidation of CLZ. In the concentration range of 0.75–100 μM of CLZ in citrate buffer solution (CBS, pH 6), the anodic peak currents, measured via differential pulse voltammetry (DPV), presented good linear relationship with limit of detection (LOD) of 2.15 nM and good sensitivity of $0.0477 \mu\text{A} \mu\text{M}^{-1}$. The proposed method was successfully applied to quantify the amount of CLZ in the blood serum and plasma of schizophrenia patient.

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1. Introduction

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Graphene, which consists of a one-atom-thick planar sheet comprising sp^2 -bonded carbon structure with exceptionally high crystal and electronic quality, is a novel material that has emerged as a rapidly rising star in the field of material science [1]. One specific branch of graphene research deals with graphene oxide (GO). This can be considered as a precursor for graphene synthesis by either chemical or thermal reduction processes. GO is an exfoliation product of graphite oxide and it can be viewed as the most important chemical derivative of graphene because of the existence of rich functional groups [2, 3]. These groups endow GO with good processability and higher chemical activity than graphene in solution, since the existing sp^3 -hybridized carbons related to hydroxyl and epoxy groups distribute on graphitic carbon regions and those related to carboxyl groups attach on the edges [4]. The functional groups attached to the graphene framework have a high chemical activity and can specifically react with some organic molecules and biomaterials, making GO an active material in biosensors [5]. Furthermore, the rich functional groups also bring a specific catalytic activity and a high electron transfer rate in organic reactions [6]. The functional groups and sheet size are proved to be the two key factors that make GO sensitive to the environment [7]. A Certain size of GONs shows different dispersing abilities in the aqueous solution at various pH values, which can be used to sieve the GONs with specific sizes [8]. Especially, deoxygenation reaction will occur when GONs come across the strong basic condition, which would influence their chemical activities [9]. There are different chemical methods in the literatures for the synthesis of GONs, for example, graphite oxide is synthesized by either the Brodie [10], Staudenmaier [11], or Hummers method [12] or some variation of these methods. All these three methods involve the oxidation of graphite to various levels. Brodie and

Staudenmaier used a combination of potassium chlorate with nitric acid to oxidize graphite, and the Hummers method involves the treatment of graphite with potassium permanganate and sulfuric acid. Graphite salts made by intercalating graphite with strong acids such as H_2SO_4 , HNO_3 or $HClO_4$ have also been used as precursors for the subsequent oxidation to graphite oxide [13]. The polar oxygen functional groups of graphite oxide render it hydrophilic, so graphite oxide can be exfoliated in many solvents, and disperses particularly well in water. Dispersions of graphene oxide platelets can be obtained by stirring and more typically by sonication of GONs in solvents [14]. All of these chemical methods need difficult experimental conditions and they are time-consuming and not beneficial. On the other hand, the efficiency of the transformation of graphite to graphene oxide by these chemical methods is very low. In recent years, electrochemical methods, because of their ease of performance, cost-effective, green and high efficiency, have attracted much attention for the synthesis of graphene oxide. Sahoo *et al.* [15] reported a simple, green and cost-effective approach for the synthesis of high-quality GO flakes via electrochemical exfoliation of pencil core in H_3PO_4 electrolyte. Kakaei *et al.* [16] reported an electrochemical method for the production of GO and graphene nanosheets using electrochemical intercalation of cetyltrimethylammonium bromide (CTAB) into graphite followed by the electrochemical exfoliation of a CTAB-intercalated graphite electrode.

Schizophrenia is a devastating mental illness that impairs mental and social functioning and often leads to the development of comorbid diseases [17]. CLZ is an effective antipsychotic drug treating positive and negative symptoms of schizophrenic patients who do not respond well to traditional neuroleptic drugs [18]. On the other hand, CLZ is an atypical antipsychotic medication with high toxicity. So the

amount of this drug must be monitored in patients and pharmaceuticals. Several analytical methods have been applied for the determination of CLZ in pharmaceutical and clinical preparations. These methods include liquid chromatography-mass spectrometry [19], liquid chromatography-tandem mass spectrometry [20], high-performance liquid chromatography [21], capillary zone electrophoresis [22], gas chromatography-mass spectrometry [23] and spectrophotometry [24]. Several electrochemical methods have been reported in the literature for the determination of CLZ, including adsorptive anodic voltammetry using glassy carbon electrode (GCE) and modified carbon paste electrode (CPE) [25], anodic stripping voltammetry on a GCE modified by the electrochemical oxidation procedure [26], cyclic voltammetry (CV) using carbon nanotubes-sodium dodecyl sulfate (CNTs-SDS) modified CPE [27], analysis of CLZ using a biosensor electrode [28], potentiometry with a novel PVC membrane electrode [29] and detection of CLZ using a gold electrode modified with 16-mercaptohexadecanoic acid self-assembled monolayer [30]. Because of low cost and less time-consuming and ease of performance electrochemical methods are good candidates for the determination of CLZ.

In this article, GONs were synthesized via electrochemical exfoliation of graphite rod and the electrochemical determination of CLZ was investigated by GONs modified graphite electrode. All the electrochemical parameters like the effect of pH of the solution, scan rate and interference effect were studied. The application of the proposed electrode was studied by determining the amount of CLZ in the serum and plasma of a patient with schizophrenia.

2. Experimental procedure

2.1. Chemicals

All the chemicals were of analytical reagent grade and used as received without further purification. Clozapine was obtained from Alfa Aesar (Ward Hill, MD, USA). Graphite rod, citric acid and sodium citrate were purchased from Merck (Darmstadt, Germany). The citrate buffer solution (CBS, pH 6) was prepared by mixing the proper amount of the solution of 0.1 M citric acid and 0.1 M sodium citrate. Double distilled water was used to prepare all solutions.

2.2. Apparatus

Electrochemical measurements were performed using the μ Autolab PGSTAT 30 electrochemical analyzer (Ecochemie BV, Utrecht, the Netherlands) coupled with a conventional three-electrode system. A saturated Ag/AgCl and a platinum wire were utilized as reference and counter electrodes, respectively, and a graphite rod modified with GO was used as working electrode. All electrochemical studies were performed at 25 ± 1 °C. The pH of the solutions was adjusted by a model 827 pH Lab Metrohm Swiss meter. A model CM10 transmission electron microscope (TEM, Philips) and LEO 1430VP model scanning electron microscope (SEM) were used to characterize the size and morphology of the GONs, orderly. Fourier transform infrared (FT-IR) spectra were recorded in KBr pellets in the frequency range of 400–4000 cm^{-1} using Tensor 27, Bruker.

2.3. Preparation of GONs modified graphite electrode (GONs/GE)

Prior to modification, the tip of the graphite rod electrode (GE) was washed with ethanol to remove any possible contaminant. Electrochemical exfoliation of the graphite rod was performed by using a multichannel electrochemical analyzer (μ Autolab PGSTAT 30) in a three-electrode cell. The graphite rod was placed as working electrode, and GONs were prepared in situ on a graphite rod electrode by using a one-step electrochemical

procedure; the counter electrode and the reference electrode were platinum and saturated Ag/AgCl, respectively. For in situ synthesis of GONs, four cycles of cyclic voltammetry scanning were run between 0 and 2.0 V at a scan rate of 50 mV s⁻¹ in 0.025 M phosphate buffer solution at pH 6.9 (Fig. 1) [31].

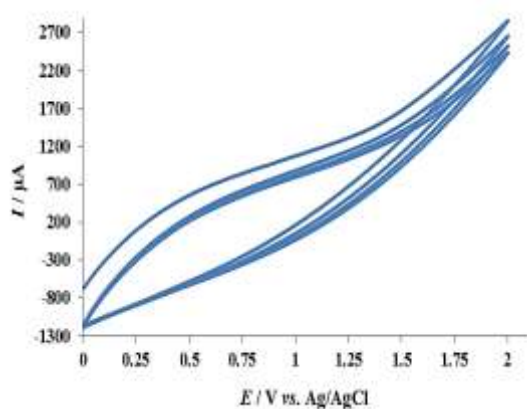


Fig. 1. Cyclic voltammograms of electrochemical synthesis of GONs from graphite rod in 0.025 M phosphate buffer solution (pH 6.9) at scan rate of 50 mV s⁻¹ (four cycles).

2.4. Preparation of real samples

Serum and plasma samples were collected from a male patient with schizophrenia in Aria Hospital (Rasht, Iran). Serum and plasma preparations were done by adding 100 μL of each to a separate volumetric flask and diluting them with double distilled water up to 100 mL.

3. Results and discussion

3.1. Characterization of GONs

Fig. 2A shows the TEM image of GONs synthesized on the surface of the graphite rod electrode. The typical wrinkle and flake of the GONs can be clearly observed in the image; it is suggested that the modified electrode has much larger internal surface per external geometric area. Fig. 2B shows SEM image of GONs. Single flakes of GO may be observed. GO flakes have relatively large surface (with the edge of sheets about the size of micrometers) and their morphology resembles a thin curtain. These parameters indicate very good exfoliation of graphite during oxidation process. Fig.

2C represents the FT-IR spectrum of GONs. The broad and intense peak at 3300–3500 cm⁻¹ is characteristic of O–H groups. The peaks at 1580 cm⁻¹, 1370 cm⁻¹ and 1060 cm⁻¹ are attributed to the C–C, C–OH and C–O stretching, respectively. The above FT-IR data indicate that there are functional groups of oxygen on the GONs surface, which resulted from anodic oxidation, hydroxylation and carboxylation of graphite electrode triggered by a positive voltage applied to electrode.

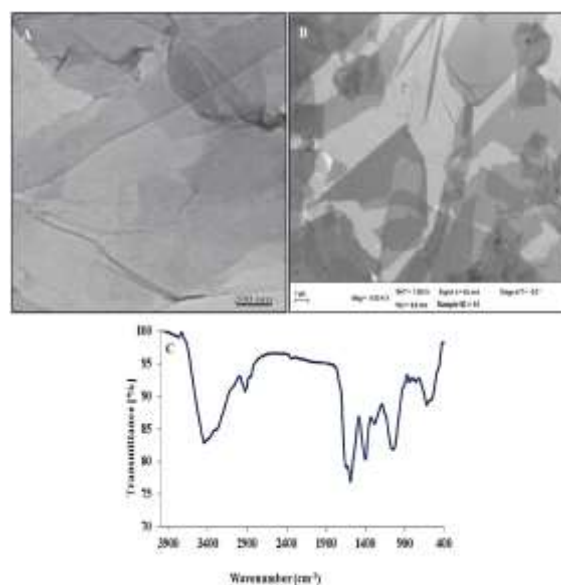


Fig. 2. TEM (A) and SEM (B) images of GONs. (C) FT-IR spectrum of synthesized GONs.

3.2 Electrochemical properties of GONs/GE

The electrochemistry of GONs/GE was characterized by CV in a mixture of 5.0 mM [Fe(CN)₆]^{3-/4-} and 0.1 M KCl. As shown in Fig. 3, a pair of well-defined redox peaks of [Fe(CN)₆]^{3-/4-} was observed on the surface of GE (solid line). The oxidation and reduction peak currents were obtained to be 701.2 and 370.3 μA, respectively. When the same solution was measured with GONs/GE under the same conditions, it was found that the oxidation and reduction peaks were sharply increased to 1852.3 and 1563.1 μA, respectively, suggesting that the electron transfer kinetics of [Fe(CN)₆]^{3-/4-} was significantly accelerated by the modified film of GONs (dotted line). On the other hand, the ΔE_p amounts for GE and GONs/GE were obtained 422 and 317 mV, orderly.

This indicated that the presence of GONs leads to ease of oxidation process of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. In order to prove this conclusion, the effective surface area of GONs/GE was calculated according to the following Randles-Sevcik equation [32]:

$$I_{pa} = (2.69 \times 10^5) n^{3/2} A D^{1/2} C^* \nu^{1/2} \quad (1)$$

where I_{pa} , n , A , D , ν and C^* are referring to anodic peak current, number of transferred electrons, surface area of electrode, diffusion coefficient, scan rate and the concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$, respectively. For 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.1 M KCl, n and D are equal to 1 and $7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, orderly [33]. At scan rate of 0.1 V s^{-1} , the active surface areas of the GE and GONs/GE were calculated to be 0.612 and 1.61 cm^2 , respectively. Therefore it can be concluded that GONs increased the active surface area of the GE.

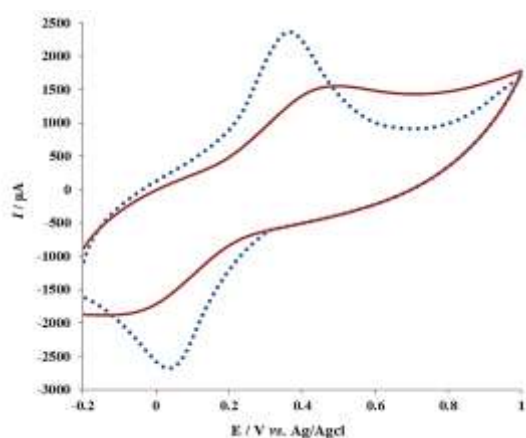


Fig. 3. Cyclic voltammograms of 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.1 M KCl on the surface of GE (–) and GONs/GE (...).

3.3 Electrochemical behavior of CLZ

The voltammetric behavior of CLZ was examined using CV. Fig. 4 shows typical cyclic voltammograms of 0.16 mM of CLZ at the surface of GE and GONs/GE in CBS (pH 6) at scan rate of 0.1 V s^{-1} . As can be seen from Fig. 4, the GONs/GE showed no behavior in the absence of CLZ. On the other hand, the GONs/GE shows much higher anodic peak currents for the oxidation of CLZ compared to GE, which indicates that the combination of GE and GONs has improved the performance of the electrode

toward CLZ oxidation. The anodic peak current of CLZ at the surface of GONs/GE is $636 \mu\text{A}$ and the amount of it for GE is $283 \mu\text{A}$. This difference is related to functional groups of GONs and higher conductivity which leads to higher sensitivity of GONs/GE toward GE.

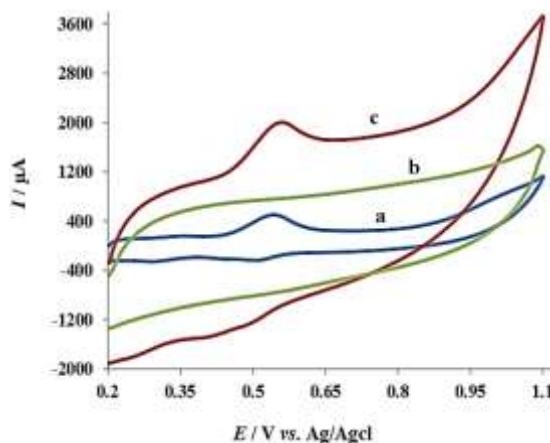


Fig. 4. Cyclic voltammogram of CLZ 0.16 mM in CBS (pH 6) at the surface of GE (a); cyclic voltammograms of GONs/GE in CBS (pH 6) (b) and (c) in CLZ 0.16 mM in CBS (pH 6) (c).

3.4. Effect of pH

Since protons took part in the electrode reaction process of CLZ, redox response of its solution must be pH dependent, so the effect of pH of solution on the voltammetric response of CLZ, on the surface of GONs/GE in CBS at pH of 4, 4.5, 5.2, 6, 6.5 and 7 containing 0.01 mM CLZ were studied by CV. As can be seen from Fig. 5, the maximum anodic peak for 0.01 mM CLZ in CBS was obtained at pH 6. Due to the high anodic peak current and relatively low anodic peak potential, a pH value of 6 was chosen for the rest of the studies. By increasing the pH of the solution, the oxidation potential of CLZ shifted to less positive potentials. As can be seen from Fig. 5, the slope of the plot of E_{pa} vs. pH is obtained -79 mV pH^{-1} ($R^2 = 0.996$), indicating that the oxidation mechanism of CLZ consists of two electrons and two protons. In other words, the number of transferred protons and electrons is equal.

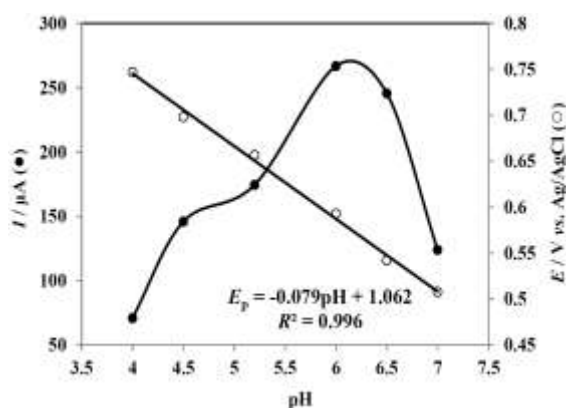


Fig. 5. The dependence of anodic peak currents (●) and anodic peak potentials (○) on the pH of the solution. Data was obtained from CVs of 0.01 mM CLZ on the surface of GONs/GE in CBS at different pH values (pH 4, 4.5, 5.2, 6, 6.5, 7).

3.5 Chronoamperometric study

The catalytic oxidation of CLZ by GONs/GE was studied by chronoamperometry. Chronoamperograms obtained at a step potential of 0.6 V are shown in Fig. 6. The mass transport limited current for the reaction of an electroactive material with a diffusion coefficient of D ($\text{cm}^2 \text{s}^{-1}$) and bulk concentration of C_b (mol cm^{-3}) is described by the Cottrell equation (eq. 2):

$$I = nFD^{1/2}AC_b\pi^{-1/2}t^{-1/2} \quad (2)$$

The plots of I vs. $t^{-1/2}$ (Fig. 6, inset *a*) were found to be linear and the slopes of these plots were used to obtain the value of D (Fig. 6, inset *b*). The mean quantity of D was found to be $5.98 \times 10^{-4} \text{ cm}^2 \text{ s}^{-1}$.

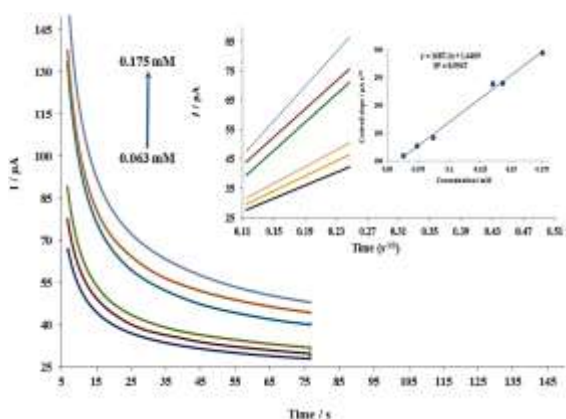


Fig. 6. Chronoamperograms of CLZ on the surface of GONs/GE in CBS (pH 6) at different concentrations (0.063, 0.074, 0.087, 0.143, 0.15, 0.175 mM) at an applied potential of 0.6 V.

3.6 Calibration curve

The dependence of the anodic peak current of CLZ on its concentrations was investigated in CBS (0.1 M, pH 6) by DPV (Fig. 7). The anodic peak currents were linearly related to the concentrations of CLZ in the range of 0.75–100 μM . The linear regression equation was $I_{\text{pa}}(\mu\text{A}) = 0.0477C_{\text{CLZ}}(\mu\text{M}) + 0.2784$ ($R^2 = 0.9961$). The LOD is computed to be 2.15 nM based on the signal-to-noise ratio of 3, and the sensitivity of the electrode was obtained $0.0477 \mu\text{M}^{-1}$. The repeatability of the GONs/GE was studied by repeating the measurement of 50 μM of CLZ. The five measurements achieved good repeatability with a relative standard deviation (RSD) of 0.88%. The reproducibility of the proposed electrode was achieved by the preparation of five similar GONs/GEs to detect the response of 50 μM CLZ and the RSD was calculated to be 2.78%. The results indicated that the GONs/GE exhibited good reproducibility in the detection of CLZ. The results obtained by the proposed electrode, GONs/GE, are much superior to that of other electrochemical sensors based on other modifiers, as shown in Table 1.

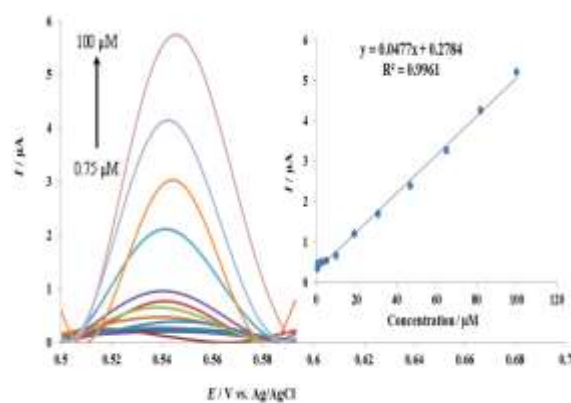


Fig. 7. DPVs obtained at the GONs/GE for CLZ in CBS (pH 6.0) at different concentrations from 0.75–100 μM . Inset: the calibration curve of CLZ.

Table 1. Comparison of various electrochemical sensors for detection of CLZ.

Electrode	Modifier	EM ^a	pH	LR ^b (μ M)	LOD (nM)	Repeatability (%)	Reproducibility (%)	Ref.
GCE	MWCNTs/NC/PPY ^c	LSV ^d	5.44	0.01–5.00	3.00	3.4	4.5	[34]
CPE ^e	TiO ₂ nanoparticles	ADPV ^f	9	0.5–45	61.0	2.0	–	[35]
CILE ^g	SDS	DPV	3.5	0.001–0.1	0.208	1.11	1.32	[36]
GCE	Silicate nanotube film	CV	–	–	–	–	–	[37]
Gold electrode	MCASM ^h	DPV	8.1	1–50	7.00	2.5	–	[30]
GE	GONs	DPV	6	0.75–100	2.15	0.88	2.78	This work

^aElectrochemical method, ^bLinear range, ^cThin film of multi-walled carbon nanotubes/new cocine doped polypyrrole, ^dLinear sweeping voltammetry, ^eCarbon paste electrode, ^fAdsorptive differential pulse voltammetry, ^gCarbon ionic liquid electrode, ^h16-mercaptohexadecanoic acid self-assembled monolayer.

3.7 Interference effect

The influence of various substances on the determination of 0.01 mM CLZ was studied by DPV. The changes in anodic peak current before and after adding different foreign interferents were measured in the 0.01 mM CLZ solution (CBS, pH 6). The tolerance limit was set as the amount of foreign ion causing $\pm 10\%$ error in the determination of CLZ. The results are tabulated in Table 2. The results showed that the presence of Na⁺, K⁺, NH₄⁺, Ca²⁺, Mg²⁺, Zn²⁺, Al³⁺, Cl⁻ and NO₃⁻ have not significantly influenced the height of the peak current. The interference of organic compounds and some coexistent substances were also determined and the tolerated concentration of the foreign substances was shown in Table 2.

Table 2. Tolerance of interferences on determination of 0.01 mM CLZ in CBS (pH 6).

Interference	Tolerance limit
Na ⁺ , K ⁺ , NH ₄ ⁺ , Ca ²⁺ , Mg ²⁺ , Zn ²⁺ , Al ³⁺ , Cl ⁻ , NO ₃ ⁻	100
Loratadine, Nortriptyline	50
Triamterene, Olanzapine, Chlordiazepoxide	20

3.8 Application

To verify the reliability of the method for analysis of CLZ in human blood serum and plasma samples, the proposed electrode, GONs/GE, was applied for the determination of CLZ in these samples. A standard

addition method was employed to evaluate the results of determination. The results are summarized in Table 3. Spectrophotometric measurements were accomplished at 280 nm as a comparative method. It should be stressed that the results obtained using the voltammetric method are in good accordance with those obtained by the spectrophotometric method. The statistical comparison of the values obtained by these methods for the determination of CLZ was done by variance ratio *F*-test and *t*-test. The statistical calculations indicate good agreement between the mean values (*t*-test) and precision (*F*-test) for the two methods (for *P* = 0.05). The standard deviations of the analysis and the recoveries of the added CLZ to the samples show that the proposed modified electrode has a capability for real sample analysis without considerable error.

Table 3. Summary of the accuracy and precision of determination of CLZ in blood serum and plasma by DPV compared with spectrophotometric method.

Sample	Added (μ M)	CLZ found (μ M)		<i>F</i> -test		<i>t</i> -test			
		Present method	Recovery (%)	RSD (%)	Reference method	Recovery (%)	RSD (%)		
Serum	–	11.17 \pm 0.04	–	2.55	10.94 \pm 0.02	–	0.04	4 (10 ⁹)	2.28 (2.78)
	10	20.02 \pm 0.07	97.5	1.88	19.12 \pm 0.00	83.14	0.12	–	–
Plasma	–	11.20 \pm 0.03	–	2.21	10.26 \pm 0.03	–	1.25	1 (10 ⁹)	2.64 (2.78)
	10	22.22 \pm 0.03	109.4	1.16	21.32 \pm 0.05	107.39	2.3	–	–

^aCLZ, for χ^2 of freedom, *F* ratio of 0.05 is 2.58.

^b*t*-test for degree of freedom, CLZ at *P* ratio of 0.05 is 19.

4. Conclusion

A popular electrochemical approach for GONs synthesis was adopted in this work so that, it saves time, creates zero explosion risk, releases no

hazardous gases, and avoids environmental pollution. Next, a facile method for analyzing the electro-oxidation behavior of CLZ was developed in this work based on the use of GONs as the sensing material. Observation of a well-defined peak and a significant increase in peak current at the GONs/GE demonstrated that the GONs could be used as an efficient promoter to enhance the kinetics of the electrochemical process of CLZ. The results indicated that the GONs/GE has good reproducibility in the detection of clozapine. Also, the results obtained by the proposed electrode, are much superior to that of other electrochemical sensors based on linear range and other criteria. The proposed electrode was successfully applied for the determination of CLZ in blood serum and plasma of a schizophrenia patient with satisfying results. Optimization of the experimental conditions yielded a detection limit and linear range similar to or better than those described in the literature. Finally, these results indicate that the GO nanosheets could be used as a promising new material for biological applications such as biosensors.

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Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this manuscript.

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