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## **Modifying a Glassy Carbon Electrode with Reduced Graphene Oxide for the Determination of Levofloxacin with a Glassy**

**Mohammad Hossein Ghanbari,<sup>1,2</sup> Mehdi Rahimi-Nasrabadi<sup>1,2,\*</sup> and Hossein Sobati<sup>3,\*</sup>**

<sup>1</sup>*Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran*

<sup>2</sup>*Faculty of Pharmacy, Baqiyatallah University of Medical Sciences, Tehran, Iran*

<sup>3</sup>*Health Research Center, Lifestyle Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran*

\*Corresponding Author, Tel.: +982182483409

E-Mail: [rahimi@bmsu.ac.ir](mailto:rahimi@bmsu.ac.ir); [Sobatih@gmail.com](mailto:Sobatih@gmail.com)

*Received: 27 September 2018 / Received in revised form: 5 January 2019 /*

*Accepted: 18 January 2019 / Published online: 28 February 2019*

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**Abstract-** The properties of a glassy carbon electrode (GCE) were modified using reduced graphene oxide (rGO) for use in the analysis of levofloxacin (LEV). The behavior of the modified GCE (mGCE) were assessed based on cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and UV-Vis spectroscopy, and its surface was studied through scanning electron microscopy (SEM) tests. Electrochemical measurements were performed using the electrode in differential pulse voltammetry (DPV) and CV experiments. The operational factors affecting the results, including the potential scan rate, the pH of the solution, were evaluated and optimized. The linear range for LEV determination were from 100 to 3000  $\mu\text{M}$  and 0.2 to 100  $\mu\text{M}$ . The limit of detection of the electrode was  $6.0 \times 10^{-8}$  M. The modified electrode by rGO demonstrated a number of advantages: a simple preparation route, high sensitivity and excellent reproducibility. The mGCE was used to determine LEV in serum samples and the results were acceptable.

**Keywords-** Levofloxacin, Electrochemistry, Sensor, Modified glassy carbon electrode, Reduced graphene oxide

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## 1. INTRODUCTION

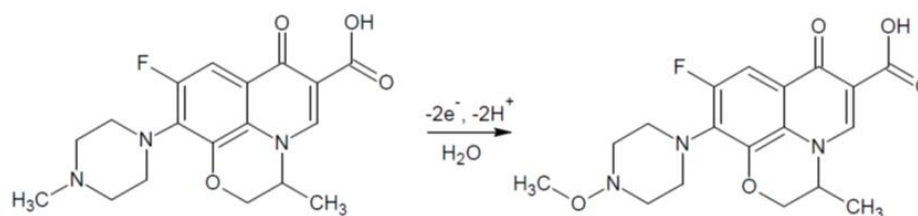
Levofloxacin ((S)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, also known as), is a fluoroquinolone antibacterial agent prescribed for gram-negative positive pathogens and bacteria, which acts based on inhibiting the DNA gyrase of these organisms [1]. Levofloxacin (LEV) is known to be effective against mycobacteria and rickettsias, and is a common medicine for genitourinary, respiratory and gastrointestinal tract infections and of the skin and soft tissue in humans and animals. Extensive administration of LEV is known to lead to increased risks of certain tendon injuries, heart diseases, and pseudomembranous colitis. The application of LEV in livestock and fish farming has enhanced the risks of its presence of in food, which might be dangerous. Excessive application of the drug might also cause resistance in bacteria [1-3].

Given the above, monitoring the levels of LEV in various biological samples is important. Knowing that LEV does not undergo considerable metabolic reactions in body and leaves the body almost unchanged through urine, various techniques, including fluorometric methods, HPLC, capillary electrophoresis, nuclear magnetic resonance, flow injection spectrophotometric methods and chemiluminescence, have been proposed and used for the analysis of LEV in urine samples [4-7]. However, most of these methods suffer drawbacks for example, they may be costly or time-consuming, lack sufficient sensitivity, or require complex sample pretreatment steps. Electrochemical sensors have recently been used for this purpose [8,9] because of their ease of operation, the simplicity of the equipment required, the reasonable costs, short analysis times and high sensitivity [10-12].

Nanotechnology has transformed the various arenas of human life and nanostructures have brought us many abilities in different technological and scientific field [13-28]. However, there is still a need for sensitive and selective analysis tools. Analytical tools and techniques such as capacitors, electronic devices and sensors have been reportedly modified through the application of different nano-structured materials [29-36]. An example of such nanomaterials used in the modification of analytical tools is graphene, a highly conductive 2D carbon nanostructure with great catalytic activity [37-39].

The Graphene oxide (GO) can be reduced to form Reduced graphene oxide (i.e. rGO through the application of fairly toxic reducing agents, or alternatively—under milder conditions and in the absence of such reagents—via electrochemical reduction [40]. Electrochemically produced rGO has been reported to have relatively strong conducting and catalytic properties [41]. However, rGOs prepared in aqueous media are known to aggregate, which decreases their effective surface area [42]. Electrochemical routes offer advantages such as moderate cost, simplicity and high speed, and the possibility of use in miniaturized applications. Conducting polymers, metal nanoparticles, selective liquid membranes, and carbon-based nanostructures have been used, to make it possible to use the technique [43-47].

Here a glassy carbon electrode (GCE) (Scheme 1) was modified using rGO. The properties of the developed rGO/GCE were studied using EIS, and SEM techniques, and it was also used for cyclic voltammetric (CV) and differential pulse voltammetric (DPV) determination of LEV.



**Scheme 1.** Proposed reaction mechanism for oxidation of LEV

## 2. EXPERIMENTAL

### 2.1. Materials and instruments

LEV and graphite powder were obtained from Merck were used as received. The supporting electrolyte was a phosphate buffer solution (PBS), which was prepared by dissolving proper  $K_2HPO_4$  and  $KH_2PO_4$  in deionized water. LEV solutions were prepared by diluting a stock  $1.0 \times 10^{-2}$  M LEV in deionized water. All materials were analytical grade pure and were not subjected to any pretreatment prior to use. Electrochemical measurements were carried out with a Palm Sens (Em state 3+) potentiostat. The electrochemical measurements were performed in a set-up composed of mGCE (or a non-modified GCE for comparative studies) as the working electrode, an Ag/AgCl/3M KCl as the reference and a platinum wire as the counter electrode. pH readings were performed using a Bel PHS3-BW pH/mV. The experiments were conducted under ambient conditions.

### 2.2. Preparation of the electrode

The non-modified GCE (nGCE) was initially rubbed with alumina powder (0.05  $\mu$ m), and then ultrasonically cleaned in deionized water for 2 min, before final ultrasonic cleaning in nitric acid and deionized water (1/1 by volume) During 1 min.

### 2.3. Fabrication of rGO/GCEs

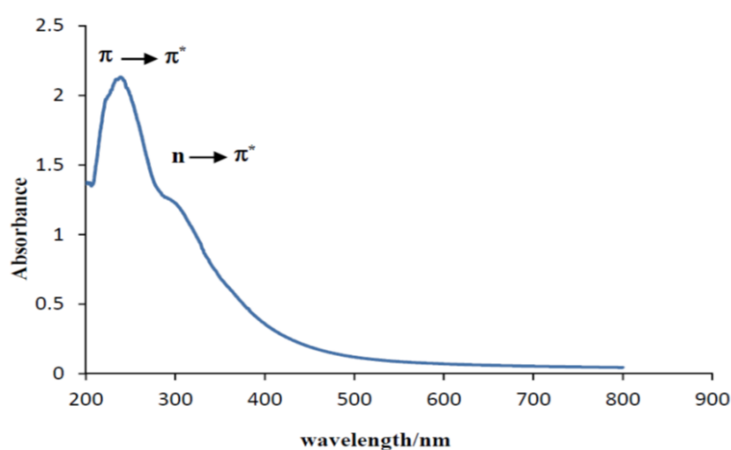
Graphene oxide was formed prepared using natural graphite in an improved version of Hummers' method [48]. Then a homogeneous suspension of 1 mg of GO in 1 mL of ethyl alcohol was prepared under sonication for 0.5 h. 5  $\mu$ L of the GO solution (0.5 mg/mL) was dropped on the surface of the nGCE and left to dry. GO was electrochemically reduced in a 0.05 M PBS (pH=5) by applying potentials of  $-1.5$  to  $0$  V at a scan rate of 100 mV/s over 100

cycles. The resulting assembly (rGO/GCE) was finally allowed to dry under ambient conditions.

### 3. RESULTS AND DISCUSSION

#### 3.1. Characterization of GO

The result of the UV-Vis spectroscopy analysis of the graphene oxide sample is shown in Figure 1. The peaks around 230 and 305 nm may be attributed to the  $\pi-\pi^*$  and  $n-\pi^*$  transitions of GO. After the reduction process, the peak at about 230 nm underwent a red shift to 265 nm, and that at 305 nm disappeared, reflecting the complete reduction of GO to rGO with a highly electronic conjugated structure [49].



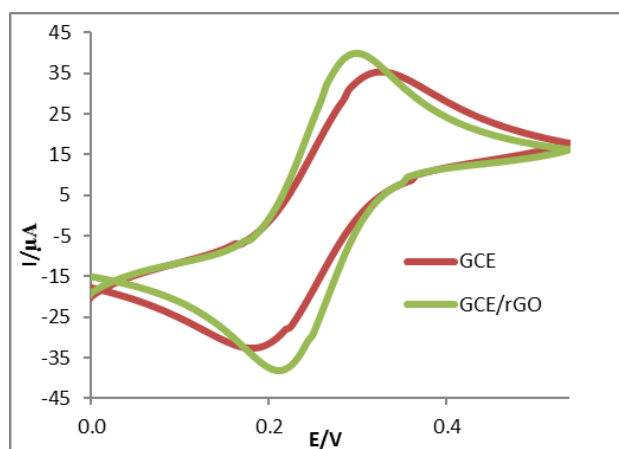
**Fig. 1.** UV-Vis spectrum of GO

#### 3.2. Characterization of rGO/GCE

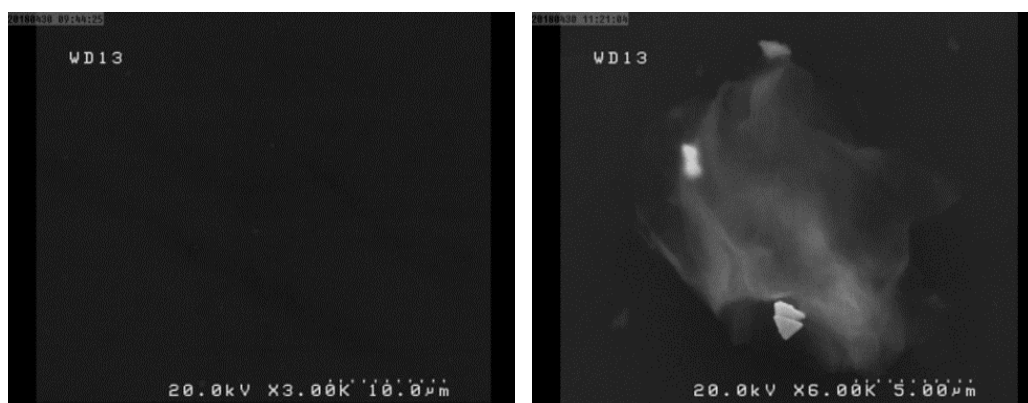
The response profile of the modified GCE in CV analyses of the  $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$  couple was monitored. The result obtained in a 5 mM 1:1 solution of the  $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$  probe in 0.1 M KCl, at 100 mV/s, is presented in Figure 2. In the case of the bare GCE, two distinct redox signals with a peak/peak separation ( $E_p$ ) of 86 mV were observed. In the case of GO/GCE the peak currents of the redox peaks clearly decrease due to the accumulation of negative carboxyl group on the surface of GO [3,43], which deters the diffusion of the also negative  $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$  species towards the surface of the electrode from the solution. Also, the decrease of 72 mV in the  $E_p$  difference in the case of the GCE /rGO indicates that the GO film improves the reversibility of the process.

The SEM images in Figure 3 illustrate the surface morphology of different GCEs. Figure 3a indicates that the unmodified GCE has an amorphous carbon morphology, while Figure 3b shows a wrinkled graphene morphology. These further confirm the deposition of rGO on the

GCE surface. Given that the rGO species are not able to easily convert back to the initial graphitic structure.



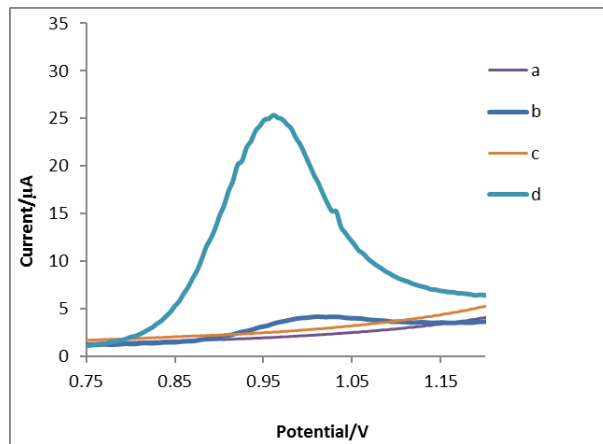
**Fig. 2.** CVs of different electrodes recorded in 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  (1:1) solution containing 0.1 M KCl at a scan rate of 100 mV/s



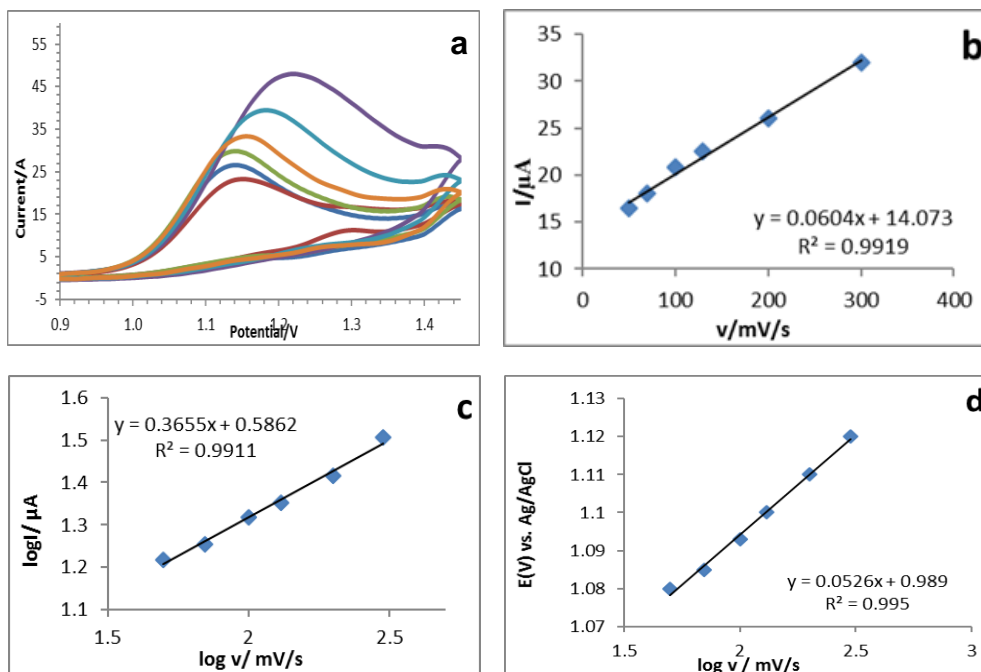
**Fig. 3.** SEM images of the working surface area for (a) bare GCE; (b) rGO/GCE and

### 3.3. Improvements of the modified rGO/GCE

The differential pulse voltammograms (DPVs) recorded at 100 mV/s in a 0.1 mM solution of LEV in PBS (pH=6.0) (Figures 4b and d) and in a blank solution not containing LEV (Figures 4a and c), using the bare GCE and rGO/GCE, are presented in Figure 4. No redox peaks were observed in the case of the blank solution, for both electrodes. The only difference between the results for the two electrodes (Figures 4a and c) was that the background current was larger for the modified GCE; this was attributed to the elevated resistance due to the different surface properties of the two electrodes and the fact that the modified electrode can be significantly changed. Figures 4b and d show that the anodic peak current observed with the LEV solution is higher in the rGO/GCE case.



**Fig. 4.** Differential pulse voltammograms of the glassy carbon electrode in PBS buffer solution with pH 6.0 at scan rate 100 mV/s, for the bare glassy carbon electrode (a and b) and rGO/GCE (c and d). Electrodes were exposed to a PBS blank solution (a and c) or 0.1 mM LEV solution (b and d)



**Fig. 5.** (A) CVs of rGO/GCE buffer solution (pH 6.0) containing  $1.0 \times 10^{-4}$  M LEV at scan rates ranging from 50 to 300 mV/s (inner to outer); (B) Variation of anodic peak current vs.  $v$  (C)  $\log I_{pa}$  vs.  $\log v$  (D)  $E$  vs.  $\log v$

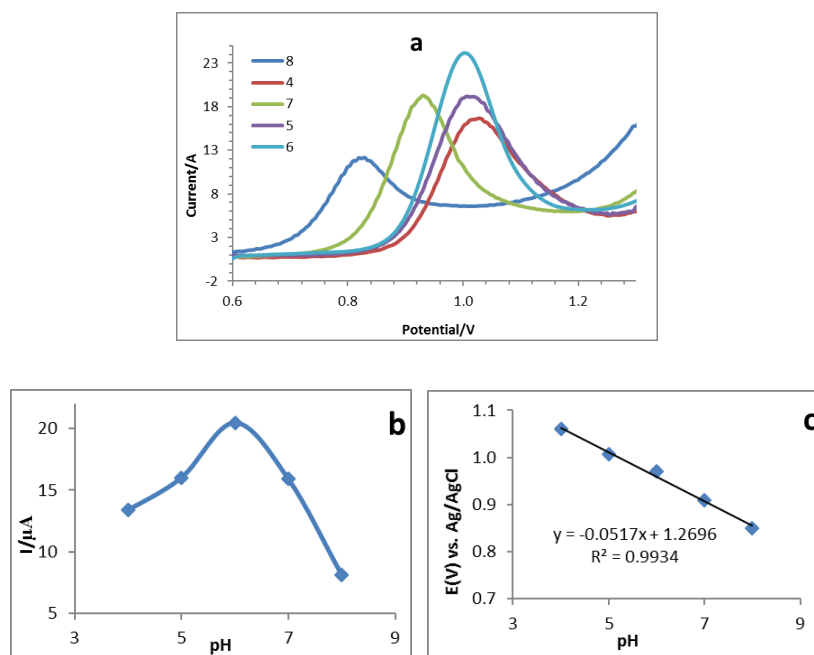
### 3.4. Effect of scan rate

The effect of the potential scan rate on the electrochemical profile of the rGO/GCE sensor was studied by recording CVs at different potential scan rates (50 to 300  $\text{mV s}^{-1}$ ) in a  $1.0 \times 10^{-4}$  M solution of LEV in 0.1 M PBS (pH=6.0) (Figure 5). The results, shown in Figure 5b, reveal

the good linearity of the plot of peak current versus the square root of the scan rate ( $\Delta i_{pa}$  vs.  $v$ ). Based on the results, it can be argued that the oxidation of LEV is a diffusion-controlled process. The slope of the plot of the logarithm of the anodic peak current ( $\log \Delta i_p$ ) against the logarithm of the scan rate ( $\log v$ ) is 0.36 for LEV, providing further evidence of this fact [50]. Furthermore, the peak current potential shifted to more positive values in the LEV solutions upon an increase in the scan rate, reflecting the irreversibility of the process (Scheme 1) [51].

### 3.5. Effect of pH

The influence of the pH of the 0.1 M PBS on the response of the solution was evaluated in the range 4.0–8.0; the resulting I/E plots are given in Figure 6. It can be seen that increasing the pH caused an increase in the peak current, with the highest current at pH 6.0 (Figure 6a). Figure 6b shows the plot of peak potential versus pH. As can be seen in Figure 6c the peak potential decreases with increasing pH from 4.0 to 8.0. This supports the finding that a chemical (i.e. proton transfer) reaction precedes the electrode process. The best peak current was observed at pH=6.0, and this value was used in the remainder of the experiments.



**Fig. 6.** Cyclic voltammograms of 200 μM LEV in PBS 0.1 M recorded from pH 4 to 8 at a scan rate of 100 mV/s(A); Effect of pH of LEV solutions on the peak current (B) and peak potential (C)

### 3.6. Performance characteristics

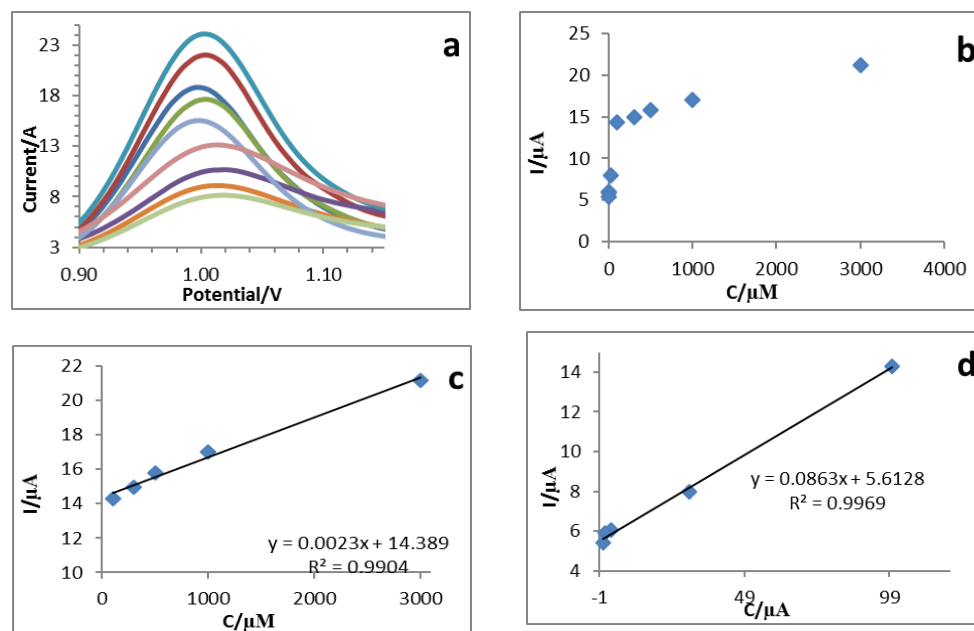


### 3.6.1. Stability and repeatability of the electrode

The modified electrode was found to produce stable results, even after 3 days when preserved in a closed container. During this period DPV voltammograms of a 0.1 mM solution of LEV were recorded every four days using the modified electrode. It was found that after this period the modified electrode produced 82.0% of its original peak current, with a standard deviation of 18%, which reflects its long lifetime. The repeatability of the results was estimated by recording six replicate DPV voltammograms in a 0.1 mM solution of LEV in PBS (pH=6.0). The relative standard deviation (RSD) was calculated to be 2.0%, which is an excellent result, indicating the suitability of the reduced graphene oxide glassy carbon electrode for applications in real analysis.

### 3.6.2. Voltammetric determination of LEV

Figure 7a shows DPV curves obtained for different LEV solutions in PBS (pH=6.0) with concentrations ranging from  $2.0 \times 10^{-7}$  to  $3.0 \times 10^{-3}$  M. The plots of  $I_{pa}$  vs. LEV concentration (Figure 7b and c) were found to be linear in the two range of  $2.0 \times 10^{-7}$  to  $1.0 \times 10^{-4}$  M ( $R^2=0.9969$ ) and  $1.0 \times 10^{-4}$  to  $3.0 \times 10^{-3}$  M ( $R^2=0.9904$ ).



**Fig. 7.** (A) Differential pulse voltammograms of rGO/GCE in various LEV concentrations in the range 0.2 to 3000  $\mu M$ ; (B) Plot of  $I_p$  vs. LEV concentration in the range 0.2 to 3000  $\mu M$ ; (C) Plot of  $I_p$  vs. LEV concentration in the range 100 to 3000  $\mu M$ ; (D) Plot of  $I_p$  vs. LEV concentration in the range 0.2 to 100  $\mu M$

The respective linear regression equations of the two linear sections of the calibration curve were  $I(\mu A)=0.0859 C(\mu M)+5.655$ , and  $I(\mu A)=0.0023C(\mu M)+14.389$ , where  $I_{pa}$  is

expressed in  $\mu\text{A}$ , and  $C$  is the concentration of LEV in  $\mu\text{M}$ . The limit of detection was  $6.0 \times 10^{-8}$  M at an S/N value of 3.

Comparison of the results with those obtained for the proposed electrode indicates that it produces comparable or even better results in terms of determination, detection limit and response linearity ranges can be observed in Table 1 [8,9,52]. The developed electrode also offers many advantages, including a low limit of detection and a rather wide response linearity range. Further figures of merit include ease of use, and repeatability and reproducibility of the results, and easy, cheap and fast application.

**Table 1.** Comparison of the performances of different electrochemical sensors

Electrode	Linear range ( $\mu\text{M}$ )	LOD ( $\mu\text{M}$ )	Ref.
DsDNA/GCE	0.5–5	0.1	[8]
MWCNT-SnO <sub>2</sub> /GC	1.0–9.9	0.2	[9]
MIP/G-AuNPs	1.0–100.0	0.53	[52]
rGO/GCE	<b>0.2-100</b> <b>100-3000</b>	<b>0.06</b>	<b>This work</b>

### 3.7. Real samples

For assessing the applicability of the mGCE in the analysis of real samples, LEV concentration in blood serum samples was determined after diluting the samples with the supporting electrolyte through the standard addition method (SAM).

**Table 2.** Determination of LEV in blood serum samples

No.	Primary( $\mu\text{M}$ )	LEV added( $\mu\text{M}$ )	LEV found ( $\mu\text{M}$ )	Recovery (%)
1	-	10.00	9.70	<b>97</b>
2	-	5.00	5.27	<b>105</b>
3	-	20.00	18.80	<b>94</b>

The results were calculated comparing the determined concentrations with the added concentrations. The RSD and recovery values (Table 2) were obtained based on five replicate analyses on three different samples. The recovery values were in the window of 94% to 105%,

demonstrating the good accuracy of the method and its potential for successful use in real sample analysis.

#### 4. CONCLUSION

A novel modified GCE (mGCE) was developed and applied for the analysis of LEV by an electrochemical method. The rGO/GCE was found to have excellent electrocatalytic activity with respect to LEV, due to the synergistic effect of reduced graphene oxide. The electrode had a low limit of detection limit, and the results were satisfactorily stable and reproducible. This makes the mGCE an excellent candidate for the analysis of LEV, which was practically proved by its successful application in the analyses of LEV contents of human blood serum samples through SAM.

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