

PREPARATION OF A MODIFIED ELECTRODE FOR THE DETERMINATION OF LEVOFLOXACIN

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ABSTRACT

In this study, modification of glassy carbon (GC) electrode with electrochemically reduced graphene oxide (ERGO) and acid treated multi-walled carbon nanotubes (A-MWCNT) was investigated for the voltammetric determination of levofloxacin (LEV) in body fluids and pharmaceutical products. Different combinations of GO, ERGO, MWCNT and A-MWCNT were searched and the best results were obtained with GC/ERGO/A-MWCNT electrode. The modified electrode showed electrocatalytic activity towards LEV oxidation by increasing oxidation peak currents 293 times. Characterizations of the modified electrode were performed with cyclic voltammetry, electrochemical impedance spectroscopy and Raman measurements. The highest oxidation peak current was observed at pH value of 2.0. The oxidation peak currents of LEV increased linearly with the concentration of LEV in the range of 0.01 μ M and 10 μ M. The detection limit of the prepared sensor was determined as 0.0063 μ M. After three weeks' storage, the prepared electrode showed high stability by preserving 93% of its original activity towards 1.0 μ M LEV. The modified electrode successfully discriminates the voltammetric response of LEV from that of other fluoroquinolone antibiotics (enoxacin, norfloxacin and ciprofloxacin). Finally, the performance of the prepared electrode was tested in the determination of LEV in human blood serum, urine and pharmaceutical samples.

Keywords: Electrochemical sensor, Graphene oxide, Levofloxacin, Multi-walled carbon nanotube, Voltammetry

1. INTRODUCTION

Levofloxacin (LEV) is a third-generation broad-spectrum synthetic antibiotic, which can be used with oral and intravenous ways. It is very effective against gram-positive and gram-negative aerobic bacteria as well as *Helicobacter pylori* [1,2]. LEV has been widely used in human and animals. Although LEV is generally good tolerated, in some instances it may cause serious side effects like tendon ruptures, heart problems and Stevens-Johnson syndrome [3]. Therefore, determination of LEV in body fluids and pharmaceutical formulations is a very important issue. Though there are many available methods for the determination of LEV, electrochemical methods can be seen promising alternatives due to their fast, simple, inexpensive, sensitive and selective characteristics [2,4–7]. While the electrochemical methods have many advantages, conventional electrodes limit their application in analytical chemistry because of their common drawbacks such as high over-potential and low surface area. Different substances were investigated to modify the conventional electrodes. In this frame, molecularly imprinted polymers [3,8], Ag nanoparticles/electrospun CeO₂-Au composite nanofibers [9], poly(o-aminophenol)/MWCNTs composite [10] modified electrodes were developed for the voltammetric determination of LEV.

Graphene and carbon nanotubes are the most widely investigated materials to increase the performance of the electrochemical methods. Graphene, composed of a one atom-thick sheet of sp² bonded carbon atoms, has been searched lately because of its unique properties such as high surface area, high conductivity and high chemical stability [11–13]. The effectiveness of graphene was tested in many different fields like supercapacitors [14], batteries [15] and solar cells [16]. Multi-walled carbon nanotubes (MWCNTs) were another popular carbon nano-materials studied last years because of their

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unusual electronic and mechanical properties. They were also tested in many fields like graphene [17–19]. Both graphene and MWCNTs have been intensively investigated as a modifier to promote electron-transfer rates at electrode/solution interfaces in electroanalytical applications. They also offer high effective surface area, which increases the sensitivity of the electroanalytical methods, in addition to higher electron-transfer rates. Moreover, they allow modifying electrode surfaces with different functional groups, which increase the affinity of analytes towards electrode surfaces. While graphene and MWCNT have been widely used in the determination of many analytes, there was little attention given into the determination of LEV.

In this study, we have investigated the modification of glassy carbon (GC) electrode with electrochemically reduced graphene oxide (ERGO) and acid treated multi-walled carbon nanotube (A-MWCNT) for the voltammetric determination of LEV in different samples such as urine, blood serum and pharmaceutical samples. The best results were obtained by the combination of ERGO with A-MWCNT. The characterization of the modified electrode was performed. The experimental variables that affect the LEV sensitivity of GC/ERGO/A-MWCNT were examined. The highest peak currents for LEV oxidation were obtained at pH value of 2.0. The prepared GC/ERGO/A-MWCNT successfully discriminates the oxidation potential of LEV from that of other fluoroquinolone antibiotics of ciprofloxacin, norfloxacin and enoxacin. The analytical application of the GC/ERGO/A-MWCNT was successfully tested in real samples.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Apparatus

Levofloxacin (LEV, 99%) and other fluoroquinolone antibiotics were obtained from Fluka. Graphene oxide (2 mg mL⁻¹ dispersion in H₂O) and multi-walled carbon nanotube (95%, OD x L = 6.9 nm x 5 μm) were purchased from Sigma-Aldrich. Ultra-pure deionized water was obtained by a Sartorius ultra-pure water system (Germany).

A conventional five-necked electrochemical cell was used in the experiments. A saturated calomel electrode (Gammry Instruments, USA) and a platinum wire were used as the reference and auxiliary electrodes, respectively. A glassy carbon (GC, 3.0 mm, BASi, USA) electrode was served as a working electrode. Electrochemical measurements were performed with an Autolab PGSTAT 100 model potentiostat (Metrohm Autolab, The Netherlands). Raman spectrums were obtained by a Raman spectrometer (Senterra; Bruker Optics GmbH, Ettlingen, Germany). A 532-nm laser line was used as an excitation source and light power was kept at 10 mW. The resolution of the laser is 3 cm⁻¹ in the spectral region of 400-4400 cm⁻¹. The signals were collected by a charge-coupled device (CCD) detector.

2.2. Preparation of GC/ERGO/A-MWCNT

Acid-activated multi-walled carbon nanotube (A-MWCNT) was prepared by reacting 50.0 mg pristine MWCNT with 4.0 M HNO₃ for 4 hours at room temperature. After that, A-MWCNT was filtered and washed with double-distilled water. A-MWCNT dispersion was prepared by dispersing 10 mg A-MWCNT in 10 mL dimethylformamide. GC surface was polished subsequently with 1.0 μm, 0.3 μm and 0.05 μm alumina powder and cleaned by sonication in methanol and deionized water for 3 min. After that, 10 μL of 1.0 mg mL⁻¹ graphene oxide dispersion was dropped on GC electrode. After drying, the graphene oxide was electrochemically reduced by potential cycling between 0.0 V and -1.5 V for 10 scans with a scan rate of 0.05 V s⁻¹ to obtain GC/ERGO. A 10 μL of 1.0 mg mL⁻¹ A-MWCNT dispersion was dropped on GC/ERGO to obtain GC/ERGO/A-MWCNT. Different modified electrodes were also prepared (Table 1), but GC/ERGO/A-MWCNT of all the studied electrodes showed the highest electrochemical response towards LEV.

Table 1. Preparation conditions of the examined electrodes

Electrode	Preparation conditions
GC/GO	10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC.
GC/A-MWCNT	10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on GC.
GC/ER-A-MWCNT	10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on GC. After that, the electrode was electrochemically reduced.
GC/(GO+A-MWCNT)	10 μL of mixture of 1.0 mg L ⁻¹ GO and A-MWCNT was dropped on GC.
GC/ER-(GO+A-MWCNT)	10 μL of mixture of 1.0 mg L ⁻¹ GO and A-MWCNT was dropped on GC. After that, the electrode was electrochemically reduced.
GC/ERGO/ER-A-MWCNT	10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC. Firstly, the electrode was electrochemically reduced. After that, 10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on GC/ERGO. Finally, a reduction step was also applied as in the first stage.
GC/ERGO/(A-MWCNT+GO)	10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC. Firstly, the electrode was electrochemically reduced. After that, 10 μL of mixture of 1.0 mg L ⁻¹ GO and A-MWCNT was dropped on GC/ERGO.
GC/ERGO/ER-(A-MWCNT+GO)	10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC. Firstly, the electrode was electrochemically reduced. After that, 10 μL of mixture of 1.0 mg L ⁻¹ GO and A-MWCNT was dropped on GC/ERGO. Finally, a reduction step was also applied as in the first stage.
ER-GC/A-MWCNT	Firstly, GC electrode was electrochemically reduced. After that, 10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on ER-GC.
ER-GC/ER-A-MWCNT	Firstly, GC electrode was electrochemically reduced. After that, 10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on ER-GC. Finally, a reduction step was also applied as in the first stage.
GC/A-MWCNT/GO	Firstly, 10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on GC. After that, 10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC/A-MWCNT.
GC/A-MWCNT/ERGO	Firstly, 10 μL of 1.0 mg L ⁻¹ A-MWCNT dispersion was dropped on GC. After that, 10 μL of 1.0 mg L ⁻¹ GO dispersion was dropped on GC/A-MWCNT. Finally, the electrode was electrochemically reduced.
Abbreviations: GC: Glassy carbon, GO: Graphene oxide, A-MWCNT: Acid-activated multi-walled carbon nanotube, ER-A-MWCNT: Electrochemically reduced acid-activated multi-walled carbon nanotube, ERGO: Electrochemically reduced graphene oxide	

2.3. Preparation of Real Samples

Blood serum samples were supplied from a local hospital and used without any further treatment. Urine samples were obtained from laboratory stuffs and filtered by a membrane filter (0.45 μm) before use. Spiked blood serum and urine samples were prepared by mixing blood serum and standard LEV solution. To prepare pharmaceutical samples (PS), ten tablets of each PS were grinded in a mortar and a suitable amount (PS-A: 0.0230 g, PS-B: 0.0144 g) was weighed. After that, 5 mL acetonitrile and 10 mL deionized water was added to dissolve LEV and the suspension was filtered. After filtration, the solution was diluted final volume of 0.1 L. An aliquot of the final solution was transferred into electrochemical cell and differential pulse voltammetry (DPV) measurements were performed.

3. RESULTS AND DISCUSSION

3.1. Comparison of the Performances of the Modifiers

Electrochemical behaviours of the modified electrodes were searched by cyclic voltammetry (CV) measurements in phosphate buffer solution (PBS) (pH: 2) containing 50 μM LEV (Fig. 1). CV of bare GC electrode showed an oxidation peak at the potential value of +1.128 V. This peak observed at potential values of +1.126 V, +1.044 V, +1.031 V and +1.049 V for GC/ERGO, GC/MWCNT, GC/A-MWCNT and GC/ERGO/MWCNT, respectively. As can be seen, GC/ERGO electrode led to a small

cathodic shift in the oxidation potential of LEV. On the other hand, GC/MWCNT, GC/A-MWCNT and GC/ERGO/A-MWCNT led to prominent cathodic shifts in the oxidation potential of LEV indicating an improvement in the electrochemical behaviour of LEV. While the GC/ERGO electrode led to a small increase in the oxidation peak current of LEV, GC/MWCNT, GC/A-MWCNT and GC/ERGO/A-MWCNT electrodes increased the peak current tremendously. The MWCNT and A-MWCNT increased the oxidation peak current of LEV 27 and 134 times, respectively. These results indicate that the acid activation of MWCNT increased the voltammetric performance of MWCNT 5 times. This may arise from the following reasons. Acid treatment removes the impurities of MWCNT that comes from the production process, which increases the conductivity of MWCNT [20,21]. Moreover, the oxidation of MWCNT during the acid treatment leads to formation of some functional groups such as carbonyl, hydroxyl and carboxylic acids on the surface of MWCNT [20,21]. These two effects (increasing conductivity and formation of functional groups) increased the voltammetric performance of MWCNT. It is important to note that the oxidation peak current of LEV increased 293 times in the presence of GC/ERGO/A-MWCNT. This result indicates that GC/ERGO/A-MWCNT increases the electrochemical oxidation characteristic of LEV. While the ERGO film found on GC surface did not increase the electrochemical behaviour of LEV very much, its presence increased prominently the voltammetric response of A-MWCNT. This may arise from the higher electron transfer rate and higher adsorption characteristics of A-MWCNT in the presence of ERGO.

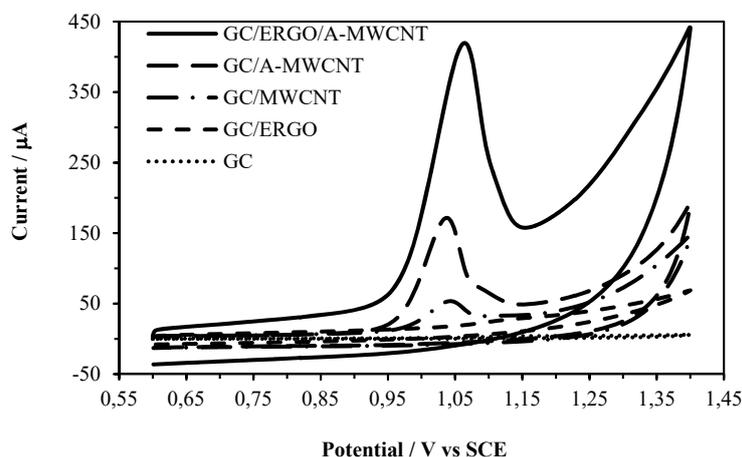


Figure 1. CVs of 50.0 μM LEV obtained with different electrodes in 0.1 M PBS (pH: 2.0). Scan rate: 0.05 V s^{-1} , accumulation time: 180 s, stirring rate: 300 rpm.

3.2. Characterizations of the GC/ERGO/A-MWCNT

Cyclic voltammograms of bare GC and the modified GC electrodes obtained in the presence of 2.5 mM $\text{Fe}(\text{CN})_6^{4-/3-}$ and 1.0 mM $\text{Ru}(\text{NH}_2)_6^{3+}$ are shown in Figure 2. An oxidation peak at the potential of +0.240 V and a reduction peak at +0.125 V were observed in CV of $\text{Fe}(\text{CN})_6^{4-/3-}$ redox couple when the working electrode was bare GC (Fig. 2A-a). A negative effect was observed at the peak potentials of $\text{Fe}(\text{CN})_6^{4-/3-}$ redox couple when the GC electrode surface was covered with ERGO (Fig. 2A-b). On the other hand, the peak currents of $\text{Fe}(\text{CN})_6^{4-/3-}$ redox couple were gradually increased when the GC/MWCNT (Fig. 2A-c) and GC/A-MWCNT (Fig. 2A-d) electrodes were used. This indicates that the effective surface area of GC electrode increases in the presence of MWCNT and A-MWCNT films. The highest peak currents were observed once the GC/ERGO/A-MWCNT electrode was used in the experiments (Fig. 2A-e). The peak separation values were calculated as 0.115, 0.595, 0.113, 0.108 and 0.137 V for GC, GC/ERGO, GC/MWCNT, GC/A-MWCNT and GC/ERGO/A-MWCNT, respectively. As can be seen, all the studied electrodes gave almost the same peak separation except GC/ERGO electrode.

The electrochemical behaviour of $\text{Ru}(\text{NH}_2)_6^{3+}$ was also investigated with the modified electrodes (Fig. 2B). A similar trend like $\text{Fe}(\text{CN})_6^{4-/3-}$ was observed in the case of $\text{Ru}(\text{NH}_2)_6^{3+}$. The highest peak currents were obtained with GC/ERGO/A-MWCNT indicating efficiency of the used modification.

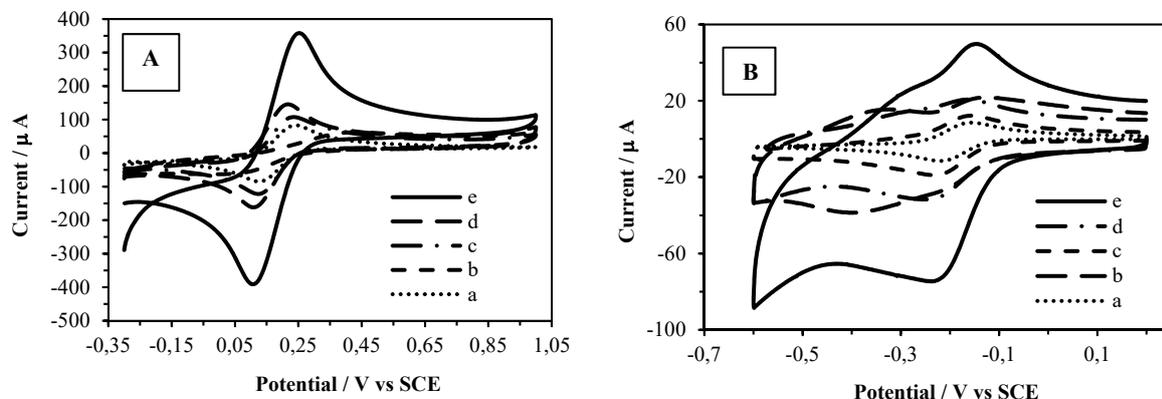


Figure 2. CVs of 2.5 mM $\text{K}_3\text{Fe}(\text{CN})_6/\text{K}_4\text{Fe}(\text{CN})_6$ (A) and 1.0 mM $\text{Ru}(\text{NH}_2)_6^{3+}$ (B) obtained with GC (a), GC/ERGO (b), GC/MWCNT (c), GC/A-MWCNT (d) and GC/ERGO/A-MWCNT (e) in 0.1 M KCl. Scan rate: 0.05 V s^{-1} .

Raman measurements were used to evaluate the formation of graphene on the electrode surface by electrochemical reduction. The Raman spectrums of GO and ERGO were given in Figure 3. As can be seen, two bands at wavenumbers of 1335 cm^{-1} and 1576 cm^{-1} were observed for both GO and ERGO. These bands correspond to characteristic D and G bands of graphene [22]. The intensities of these bands were prominently increased after electrochemical reduction indicating the increase of graphene structure on the electrode surface.

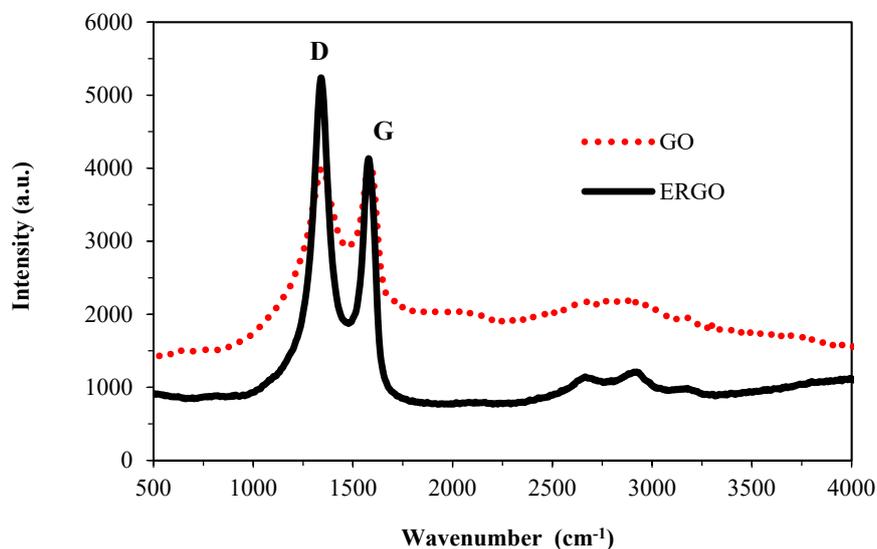


Figure 3. Raman spectrums of GO and ERGO obtained with electrochemical reduction via cyclic voltammetry measurements between the potential values of 0.0 V and -1.5 V with a scan rate of 0.05 V s^{-1} for 10 scans.

3.3. Optimization of the Parameters

The effect of pH was examined by performing DPV measurements of $5.0 \mu\text{M}$ LEV with GC/ERGO/A-MWCNT at different pH values. The oxidation peak currents decreased tremendously by the increase of pH from 2.0 to 7.4. A small increase was seen at pH value of 8.5. The highest oxidation peak current

of LEV was obtained at pH value of 2.0. Therefore, this pH was selected as the optimal pH. Different pH values such as 4.5 [10], 5.5 [9] and 6.0 [21] were also reported as the optimal values in the presence of different modified electrodes. These results indicate that the GC/ERGO/A-MWCNT showed different characteristics from the previously reported modified electrodes.

The effect of accumulation time on the oxidation peak currents of LEV was also examined. A gradual increase was seen in the oxidation peak currents when the accumulation time was increased from 30 s to 180 s. After this time, further increase of accumulation time did not lead to any meaningful increase in current values.

3.4. Investigation of the Effects of Interfering Substances

DPV measurements of LEV were performed with GC/ERGO/A-MWCNT in the absence and presence of some possible interfering substances to investigate the effects of these substances. First of all, the effects of structurally related substances such as ciprofloxacin, enoxacin and norfloxacin were searched. Differential pulse voltammograms of 2.5 μM LEV in the absence and presence of two-fold excess ciprofloxacin, enoxacin and norfloxacin were recorded (Fig. 4). As can be seen, the GC/ERGO/A-MWCNT successfully separate the oxidation potential of LEV from those of enoxacin, norfloxacin and ciprofloxacin. These results indicate that the GC/ERGO/A-MWCNT allows the selective determination of LEV in the presence of other fluoroquinolone antibiotics. This is a very important property because these substances have very similar structures with LEV. The effects of possible interfering substances (ascorbic acid, citric acid, paracetamol and tartaric acid) were also evaluated. The results indicate that the GC/ERGO/A-MWCNT electrode can be safely used in the determination of LEV in the presence of these substances.

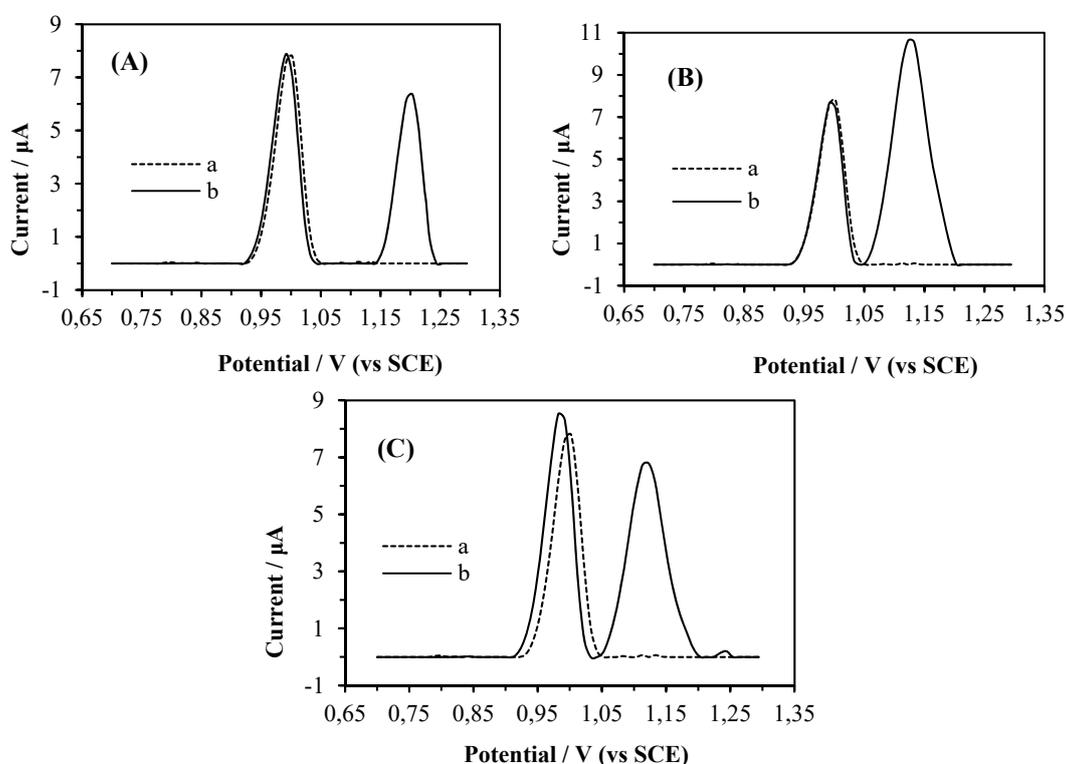


Figure 4. DPVs of 2.5 μM LEV in PBS (pH: 2.0) in the absence (a) and presence (b) of 5.0 μM enoxacin (A), ciprofloxacin (B) and norfloxacin (C). Accumulation time: 60 s, Stirring rate 300 rpm.

3.5. Stability, Reproducibility and Regeneration of GC/ERGO/A-MWCNT

Stability of GC/ERGO/A-MWCNT was tested by performing DPV measurements of 5.0 μM LEV over three weeks. The electrodes were stored in a desiccator during this period. The electrode kept 91% of its initial activity towards LEV after a 20-day storage. These results indicate that the modified electrode can be stored for a long time without any significant activity loss.

Reproducibility of the GC/ERGO/A-MWCNT electrode was investigated in three headings; intra-day, inter-day and fabrication reproducibility. Differential pulse measurements were performed with GC/ERGO/A-MWCNT electrode ten times in a solution containing 1.0 μM LEV. The relative standard deviation (RSD) of these measurements was calculated as 2.92% which represents intra-day reproducibility. To determine inter-day reproducibility, differential pulse measurements were repeated with the same GC/ERGO/A-MWCNT electrode for ten days. The RSD value of inter-day measurements was obtained as 3.45%. The fabrication reproducibility of GC/ERGO/A-MWCNT electrode was tested by preparing ten different electrodes and determining their voltammetric responses. The RSD of the measurements was determined as 4.26%. The results obtained indicate that the GC/ERGO/A-MWCNT electrode produces very reproducible results.

The regeneration of the electrode surface was also investigated. For this purpose, DPVs of 5.0 μM LEV in PBS (pH: 2.0) was performed (Fig. 5a). After that, the electrode was placed in a blank PBS (pH: 2.0) and DPV measurement was repeated (Fig. 5b). A very small oxidation peak which belongs to LEV was noticed in blank DPV measurement (Fig. 5b). This indicates that there was an adsorption of LEV on the electrode surface, but the amount of this adsorption was very small. In a separate experiment, firstly, DPV measurement of 5.0 μM LEV was performed. After that, the electrode was waited in a PBS (pH: 5.0) under stirring conditions. After 2 min, the electrode was placed in a blank PBS (pH: 2.0) and DPV measurement was done (Fig. 5c). As can be seen, no meaningful LEV signal was observed in blank measurement. This indicates that the electrode can be easily regenerated by waiting in PBS (pH: 5.0) for 2 min. This property is very important for the modified GC electrodes because one of the most important problem in modified electrodes is the regeneration of the electrode surface.

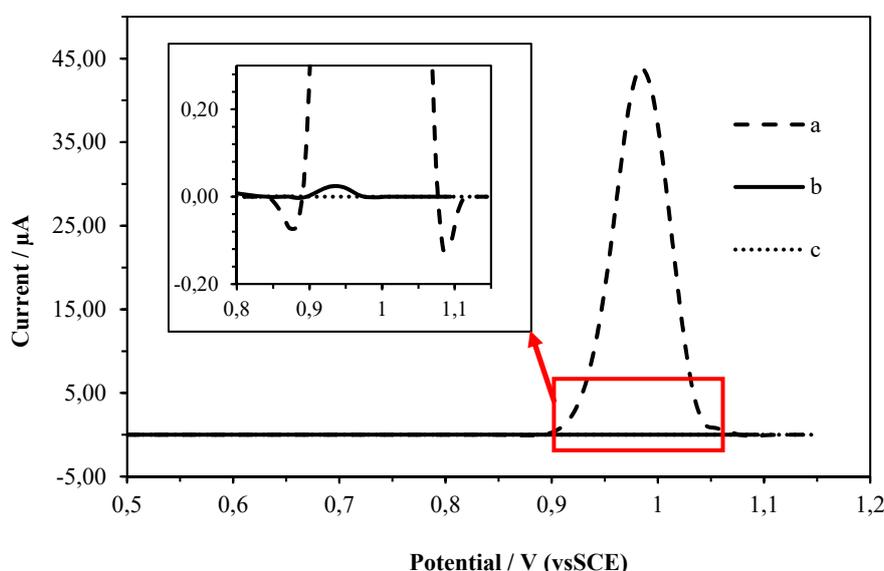


Figure 5. a) DPV of GC/ERGO/A-MWCNT in PBS (pH: 2) containing 5.0 μM LEV. b) DPV of GC/ERGO/A-MWCNT in PBS (pH 2) after waiting the electrode in deionized water for 2 min after performing the measurement a. c) DPV of GC/ERGO/A-MWCNT in PBS (pH 2) after waiting the electrode in PBS (pH 5.0) for 2 min after performing the measurement a. Accumulation time: 180 s, stirring rate: 300 rpm.

3.6. Determination of Analytical Parameters

Analytical parameters of the GC/ERGO/A-MWCNT were determined in a solution containing different amount of LEV. A linear region was observed between the oxidation peak currents of LEV and its concentrations. The corresponding line has an equation of $I_p (\mu A) = 8.80 C_{LEV} (\mu M) + 0.72$ in the concentration range of 0.01-10.0 μM . Limit of detection (LOD) and limit of quantification (LOQ) values of the method proposed in this study were calculated as 0.0063 μM (S/N: 3) and 0.021 μM (S/N: 10), respectively. Analytical parameters of the GC/ERGO/A-MWCNT were compared in Table 2 with those of other modified electrodes. The modified electrode showed superior performance than the previously reported modified electrodes.

Table 2. Comparison of the performances of various modified electrodes

Electrode type	Linear range	LOD (μM)	LOQ (μM)	Method	Reference
Molecularly imprinted polymer/graphene-gold	1-100	0.53	1.77	DPV	[3]
Silver nanoparticles-cesium oxide-gold-glassy carbon	0.03-10	0.01	0.033	DPV	[9]
Poly(aminophenol)/MWCNTs	3.00-200	1.00	3.33	DPV	[10]
Single-strand DNA-SWCNT-gold	1-10	0.075	0.25	SWV	[23]
MWCNTs-poly(alizarin)	5.00-100	0.40	1.33	LSV	[24]
Double-strand DNA-glassy carbon	0.5-5	0.10	0.33	SWV	[25]
Poly(diallyldimethylammonium chloride)-reduced graphene oxide-gold nanoparticles	10 - 200	3.90	13.00	LSV	[26]
GC/ERGO/A-MWCNT	0.01-10	0.0063	0.021	DPV	This work

3.7. Analysis of Real Samples

The determination of LEV in urine, blood serum and pharmaceutical samples was investigated with GC/ERGO/A-MWCNT to evaluate the applicability of the modified electrode in real samples. Firstly, the performance of the GC/ERGO/A-MWCNT was tested in pharmaceutical samples. Two different pharmaceutical samples were taken and their LEV contents were determined. DPVs of the first sample were given in Figure 6. As can be seen, an oxidation peak was observed at +0.968 V corresponding to the LEV. The intensity of this peak increased after each addition of standard LEV solution (Fig. 6). The second sample was also analysed in the same way. Their LEV contents were determined as 0.4900 g tablet⁻¹ and 0.4920 g tablet⁻¹. These values were very close to their initial values of 0.5000 g tablet⁻¹ indicated by the producers. Analytical application of the GC/ERGO/A-MWCNT was also tested in blood serum and urine samples. The recovery analysis was performed with these samples because they initially did not contain any LEV. The analysis of spiked blood serum and urine samples were performed by standard addition method. The recovery values obtained during these analyses were given in Table 3. As can be seen, satisfactory results were obtained in the case of both samples. These results indicate that the GC/ERGO/A-MWCNT can be safely used in the determination of LEV in such kind of samples.

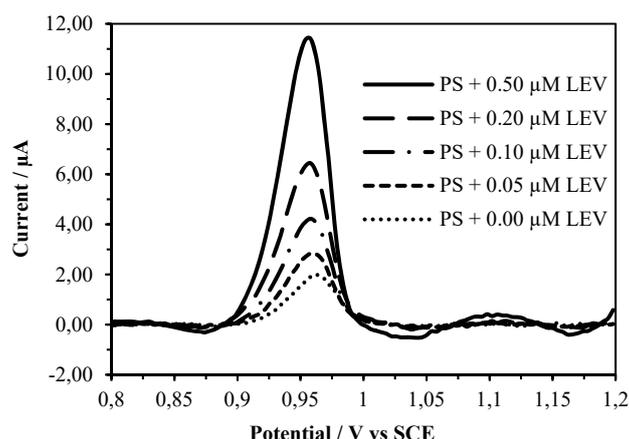


Figure 6. DPVs of GC/ERGO/A-MWCNT in PBS (pH: 2) containing thousand-time diluted pharmaceutical sample in the presence of increasing concentrations of LEV. Accumulation time: 180 s, stirring rate: 300 rpm.

Table 3. The results of the determination of LEV in spiked blood serum and urine samples

Sample	Spiked value / μM	Determined value / μM	% Recovery
BS-A	0.200	0.197	98.5
BS-B	0.500	0.5125	102.5
US-A	0.400	0.389	97.25
US-B	1.000	1.097	109.7

4. CONCLUSIONS

The application of carbon nanomaterials (GO and A-MWCNT) was investigated at different combinations to prepare an electrochemical LEV sensor. GC/ERGO/A-MWCNT electrode showed the highest oxidation peak current in the oxidation of LEV among the studied combinations. Different parameters were searched to increase the sensitivity of the modified electrode. The electrode showed superior performances according to GC in the electrochemical oxidation of LEV by increasing peak currents tremendously and discriminating the oxidation potential of LEV from other substances. The real sample analysis with GC/ERGO/A-MWCNT gave satisfactory results. According to obtained results, it can be concluded that the GC/ERGO/A-MWCNT electrode is a good candidate for the determination of LEV because of its properties such as simplicity, easy preparation, high reproducibility and high sensitivity.

ACKNOWLEDGEMENTS

This study was supported by the Anadolu University Scientific Research Projects Commission under the grant number of 1605F334.

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