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A nanocomposite consisting of reduced graphene oxide and electropolymerized β-cyclodextrin for voltammetric sensing of levofloxacin

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Abstract

A glassy carbon electrode (GCE) was modified with a nanocomposite prepared from polymerized β-cyclodextrin (β-CD) and reduced graphene oxide (rGO). The modified GCE is shown to enable the voltammetric determination of traces of levofloxacin (LEV) by various electrochemical techniques. Experimental factors affecting the results including the amount of the substrates in preparation of the nanocomposite, accumulation time, the scan rate and pH value of the electrolyte were optimized. The modified GCE, best operated at a working potential of 1.00 V (vs. Ag/AgCl), has two linear response ranges, one for low LEV concentrations (100 pmol L^{-1} to 100 nmol L^{-1}), and one for higher LEV concentrations (100 nmol L^{-1} to 100 μmol L^{-1}). The limit of detection and sensitivity are calculated to be 30 pmol L^{-1} and 467.33 nA μmol L^{-1} cm^{-2}, respectively. The modified GCE demonstrates a number of advantages such as high sensitivity and selectivity, low LOD, excellent reproducibility, high surface-to-volume ratio, and good electrocatalytic activity towards LEV. The sensor was successfully applied to the determination of LEV in spiked human serum samples.

Keywords Antibacterial agent · Electropolymerization · Nanocomposite · Electrochemical sensing

Introduction

Antibiotic-resistant bacterial infections are encountered in clinical practice due to overprescribing and overuse of antibiotics in human and veterinary medicine. Levofloxacin (LEV) (Scheme 1a) as a fluoroquinolone antibacterial agent performs activity against both Gram-positive and Gram-negative bacteria through inhibition of their DNA gyrase. This is commonly applied to treat some sensitive infections of the respiratory and urinary tract, soft tissues and skin. Widespread utilizing the LEV in livestock farming and aquaculture has also led to the presence of its residues in food sources. This may be directly toxic or cause pathogen resistance and possible allergic hypersensitivity reactions in humans [1, 2]. Consequently, the development of a valid analytical method for LEV detection is of paramount importance.

LEV detection strategies are trended toward techniques based on electrochemical methods. These methods have some advantages such as selective response, low cost, miniaturization, and simple instrumentation [3]. Sensitivity and stability

Highlights
• A sensor is based on a modified glassy carbon electrode with a nanocomposite of β-cyclodextrin and reduced graphene oxide.
• A clever combination of the substrates in the nanocomposite has some properties such as high surface to volume ratio and good electrocatalytic effect.
• LEV detection was performed in a range of 100 pmol L^{-1} to 100 μmol L^{-1} with a LOD of 30 pmol L^{-1}.
• The sensitivity value was estimated to be 467.33 nA μmol L^{-1} cm^{-2}.
• Minimum substrates and materials were handled for a possible point of care assays.

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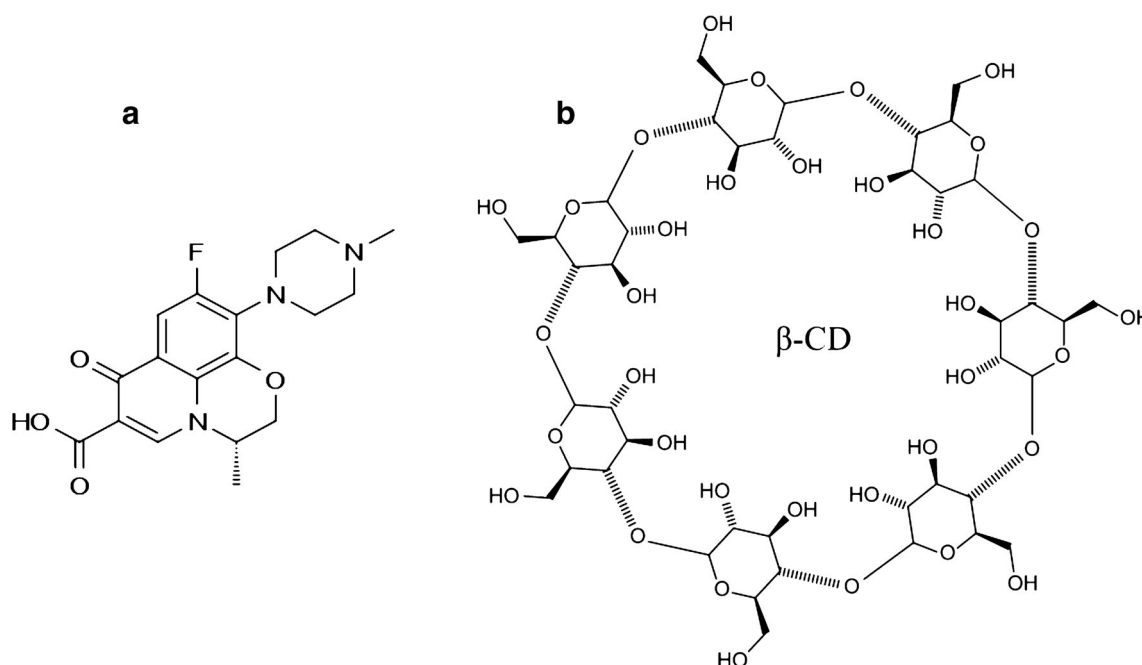
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Scheme 1 Chemical structures of the LEV and β-CD.

are key factors in the improvement of sensors performance. Normally, the signal amplification in the electrochemical sensors can be achieved using various nanomaterials [4, 5]. Zhao et al. fabricated a GCE modified with a poly(ortho-aminophenol)/multi-walled carbon nanotubes (PoAP/MWCNTs) nanocomposite for the LEV determination. Linear range was calculated to be 3.00–200.00 mol L^{-1} with a detection limit (LOD) of 1.00 mol L^{-1} [6]. A sensor based on MWCNTs modified with SnO_2 rods was reported by Cesarino et al., for measurement of the LEV levels. A linear range of 1.00–9.90 μmol L^{-1} with a LOD of 0.20 μmol L^{-1} was obtained [7]. Recently, graphene-based nanocomposites have opened a new window to design of new generation of electrodes in electrochemical detection. Reduced graphene oxide (rGO) has unique properties in electrode modifying process [8]. Zhang et al. were introduced a LEV sensor based on a molecularly imprinted polymer (MIP) incorporation with graphene–Au nanoparticles (G-AuNPs). Linear range from 1.00 to 100.00 μmol L^{-1} with a LOD of 0.53 μmol L^{-1} was reported [9]. Despite the accuracy and reliability of these methods, a majority of them is suffered from limitations such as wide linear range and low LOD. So, a sensitive sensor for LEV detection with better figures of merit still faces challenges.

β-Cyclodextrin (β-CD) (Scheme 1b) as a natural cyclo-oligosaccharide has an inner hydrophobic cavity and an outer hydrophilic shell with seven glucose molecules [10]. The structure of the β-CD enables to incorporate molecules with appropriate size into its cavity.

This incorporation can form stable host-guest inclusion complexes through some interactions such as non-covalent van der Waals, hydrophobic, electrostatic interactions or hydrogen bonding. This inherent properties of the β-CD have been widely evaluated as a modifier for electrodes in selective detection of electroactive molecules [11]. It seems that a combination of the rGO and β-CD may be a good choice to form a promising nanocomposite with high supramolecular recognition capabilities as well as enhancing conductivity. The objective of the present study is to investigate of applicability of the β-CD/rGO nanocomposite on the surface of a glassy carbon electrode (GCE) in measuring of the LEV. To the best of the authors' knowledge, no report has been introduced yet on electrochemical detection of the LEV based on the β-CD/rGO nanocomposite. Applicability of the GCE modified with the nanocomposite was evaluated for LEV sensing in human blood serum with acceptable results.

Experimental section

Materials and reagents

β-CD, graphite powder, LEV and all other materials with analytical grade were ordered from Merck (http://www.merck.com) or Sigma-Aldrich (http:// www.sigmaaldrich.com) Companies. All reagents were used without any treatments to use. An electrolyte of phosphate buffer (PB) was

fabricated using Na_2HPO_4 and NaH_2PO_4 from Sinopharm Chemical Reagent Company (http://en.reagent.com.cn/). All solutions were prepared with deionized water. The LEV was dissolved in distillated water and a solution of 1 mmol L^{-1} LEV as a stock solution was obtained.

Instruments

Electrochemical measurements were conducted using a Palm Sens Potentiostat (Em stat 3+, Netherlands) driven with PS Trace 4.8 software. The instrument was in connection with a conventional three-electrode setup. A modified or unmodified GCE as a working electrode, an Ag/AgCl (3.00 mol L^{-1} KCl) reference and a Pt wire as an auxiliary electrode (Azar Electrode, Iran) were used. Some electrochemical techniques such as electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques were applied in the following experiment. The EIS measuring was done with Ivium instrument (Compact stat, Netherlands). The DPV experiment was performed at E_{step} = 5.00 mV, E_{pulse} = 60.00 mV, t_{pulse} = 0.02 s with scan rate at 100.00 mV s^{-1}. Furthermore, the EIS experiment was recorded at a frequency range of 0.10–1.00 $\times 10^5$ Hz with 5.00 mV amplitude. The structure and morphology of the substrates were characterized using X-Ray Diffraction (XRD) taken on a Philips (X\'Pert Pro, Netherlands). Scanning electron microscopy (SEM) images were recorded by using JSM-6700F (Japan). The spectrum of ultraviolet-visible spectroscopy (UV-Vis) and Fourier Transform infrared spectroscopy (FT-IR) was obtained via Perkin Elmer/Lambda 25 and Perkin Elmer/RXIFT-IR (USA), respectively. A pH/mV meter (Bel PHS3-BW, Italy) was applied for pH measurement.

Preparation of the real samples

In our country (Iran), there is no laboratory for LEV detection. So, we have to use spiked samples of human blood serum. For the preparation of the blood serum sample as the real sample following steps were performed: Firstly, some healthy people's serum specimen was supplied by a local hospital at Tehran city. Then, the samples were subjected to ultrafiltration by loading them into a centrifugal filtration tube at 5000 rpm for 30 min. Afterward, the samples were diluted with 0.10 mol L^{-1} PB (pH = 5.00) at a ratio of 1:2. 10.00 mL LEV with a certain concentration was spiked to a certain volume of the diluted serum samples in a volumetric flask. Finally, 10.00 mL of each solution was pipetted separately and independently from the others in the electrochemical cell to test.

## Fabrication of the β-CD/rGO nanocomposite on the GCE surface

Typically, a bare GCE was polished with emery paper with 0.05 μm alumina powder. Then the GCE was briefly sonicated in a bath of deionized water and nitric acid (1:1) to remove some probably absorbed particles. After the cleaned GCE was washed with deionized water, dried at room temperature to use.

A customized version of Hummers' method [12] was used to the synthesis of the GO from natural graphite. A homogenized suspension of the GO was prepared through adding 5.00 mg GO to 1.00 mL of a mixture of ethanol and H_2O_2 (1:1) under sonication for 30 min. First, 3.00 μL of the obtained GO solution was dropped onto the bare GCE surface and air-dried freely at ambient temperature. By applying a potential range between −1.50 to 0.00 V (100 cycles) with a scan rate of 100.00 mVs^{-1} in 0.05 mol L^{-1} PB (pH = 5.00), the GO onto the GCE surface was electrochemically reduced. So, the rGO/GCE was achieved [13]. The β-CD was electropolymerized onto the rGO/GCE surface through applying a potential range between −2.00 to 2.00 V (8 cycles) with a scan rate of 100.00 mVs^{-1} in 0.10 mol L^{-1} PB (pH = 5.0) containing 0.01 mol L^{-1} β-CD [11, 14, 15]. Finally, the fabricated β-CD/rGO/GCE electrode was washed and dried to use as the sensor for the following experiment. The fabrication steps of the sensor and the performance of the LEV sensing are schematically represented in Scheme 2.

Results and discussion

Choice of materials

There are many nanomaterials and different nanocomposite to modify an electrode surface. A key factor in electrode modifying is utilizing non-toxic and economical reagent to prepare a simple method to detect the targets. The rGO which is usually achieved using electrochemical reduction (under a condition without any toxic reducing agents) [8] has been reported to have fantastic properties. This nanomaterial has relatively strong conducting and catalytic properties as well as, simplicity and the possibility of use in miniaturized applications [16]. Furthermore, the rGOs in aqueous media may aggregate on the electrode surface and lead to a decrease in the effective surface area [17]. On the other hand, some nanocomposites are reported by hybridization of the cyclodextrin and graphene to combine the excellent supramolecular sensing potentials of them in the detection of various targets [18, 19]. However, these studies are based on the covalent bonding of the components. So, this may limit the extent of cyclodextrin immobilization and consequently impairs the material's supramolecular recognition ability. Also, various studies have focused on developing new
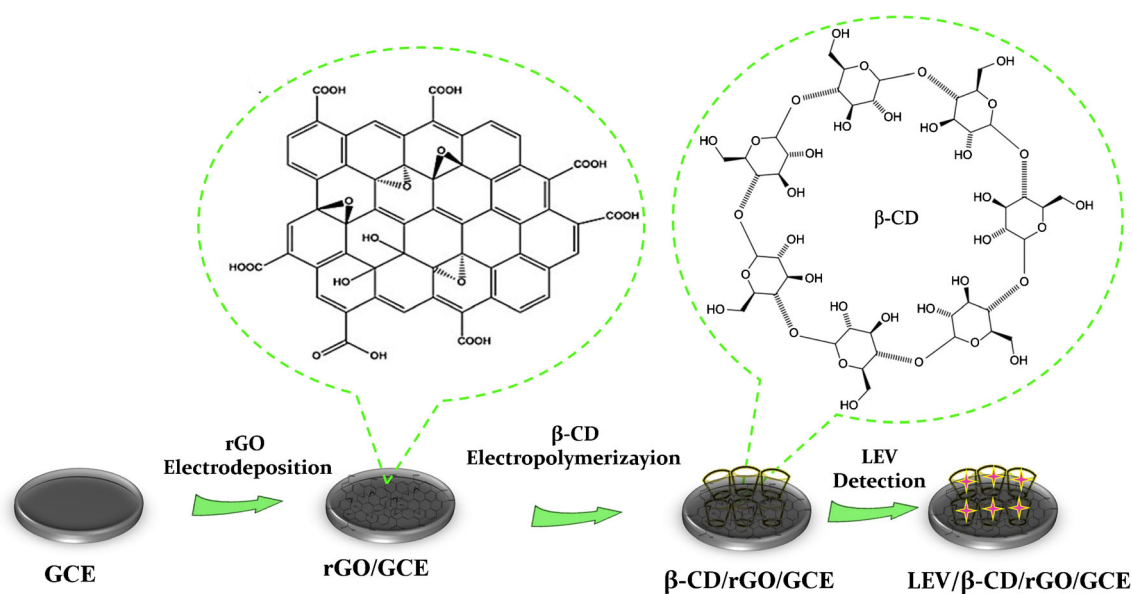
Scheme 2 Fabrication steps of the β-CD/rGO/GCE as the LEV sensor.

methods for preparing cyclodextrin/graphene hybrids with specific properties [10]. These studies show the β-CD can act as a molecularly imprinted polymer (MIP) with specific ability in the detection of various targets. Among many nanomaterials, the clever combination of the rGO and β-CD as a novel nanocomposite by preparation specific templates like the MIP may create a novel and efficient platform in the detection of the LEV.

Characteristics of the synthesized GO

The UV-Vis spectra of the synthesized GO is shown in Fig. 1a. The peaks around 230.00 and 305.00 nm are attributed to the $\pi - \pi^*$ and $n - \pi^*$ transitions of the GO, respectively. After the reduction process, the peak at about 230.00 nm underwent a red shift to 265.00 nm, and a peak at 305 nm disappeared. According to the literature, these changes reflecting the complete reduction of the GO to rGO with a highly electronic conjugated structure (data is not shown) [20]. Furthermore, the GO nanosheet was investigated with the FT-IR technique (Fig. 1b). According to Fig. 1b, the presence of the absorption band at 3340 cm^{-1} is related to the O-H group stretching vibrations. The absorption peak at 1730.00 cm^{-1} and 1630.00 cm^{-1} can be related to C=O stretching of the carboxylic and/or carbonyl moiety functional groups. Also, the two absorption peaks at about 1226.00 cm^{-1} and 1044.00 cm^{-1} are indicated the C–O stretching vibrations. These funding confirm successful synthesize of the GO [21]. Further proof for confirming of the GO was achieved by XRD pattern to determine the average crystalline properties of the GO sheet (Fig. 1c). A very strong peak at $2\theta = 11.24°$ was obtained which is in good agreement with the literature [22].

Investigation of characterization of the β-CD/rGO-modified GCE

Obviously, modification of the electrode with the β-CD/rGO nanocomposite plays a vital role in designing the sensor. Also, the electropolymerization step of the β-CD onto the rGO/GCE surface is an important step. The CV technique was applied to prepare the nanocomposite. It can be seen from Fig. 1S that the current signal is increased upon applying more potential cycles. This signal increment is attributed to the presence of the conductive β-CD film onto the rGO/GCE surface [23]. Furthermore, the oxidation peak at the potential of +0.60 V indicates the production of cationic radicals of the β-CD. These radicals can combine by losing two H$^+$ ions, the repetition of which process leads to the polymerization. Furthermore, the CV technique was used to confirm the layer by layer immobilization of the modified electrode. Voltammogram of each modified electrode was recorded in a solution of 5.00 mmol L^{-1} [Fe(CN)$_6$]$^{3-/4-}$ and 0.10 mol L^{-1} KCl (1:1) as a redox probe (Fig. 2a). As shown in Fig. 2a, two well-defined redox peaks with a peak-to-peak separation (ΔE_p) of around 86 mV are observed for the bare GCE. For the GO/GCE, a significant decrease in the peak currents was obtained. This current decrease may be attributable to the presence of the -COO$^-$ groups on the GO sheets onto the electrode surface [17]. These negatively charged groups onto the electrode surface repel [Fe(CN)$_6$]$^{3-/4-}$ species and hinder diffusion of the redox probe to the electrode surface. By adding the rGO onto the GO/GCE surface, the ΔE_p value was decreased to 72 mV. This decrement behavior indicating the presence of the rGO film enhances the reversible nature of the electrochemical reaction [24]. After coating the film of the β-CD polymer on the rGO/GCE surface, the electron transfer

Fig. 1 **a** UV-Vis spectrum, (**b**) FT-IR and (**c**) XRD pattern of the synthesized GO
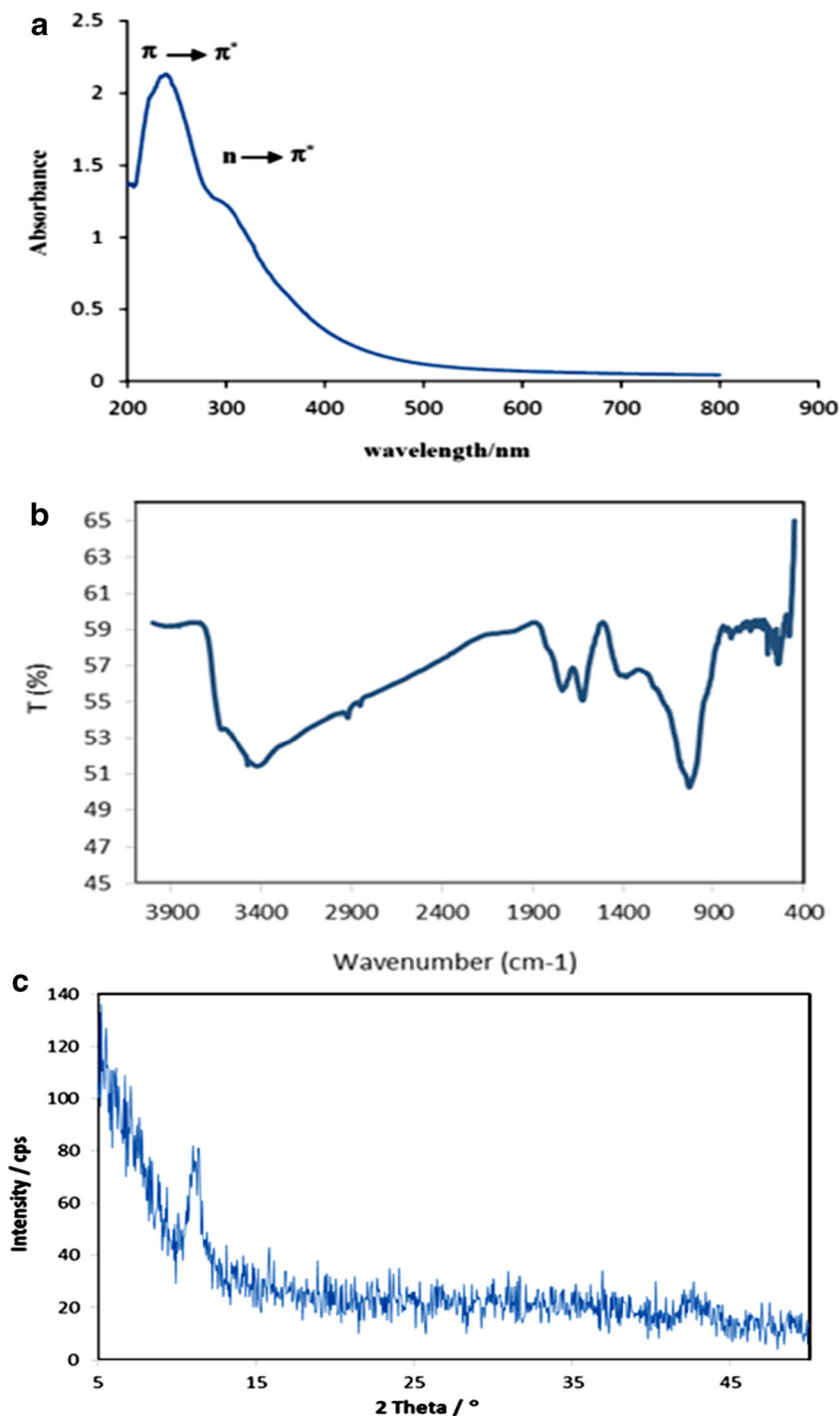
resistance was increased and the current was dramatically decreased [24]. In order to study the charge transfer resistance (R_{ct}) parameter, Nyquist plots in the redox probe in a frequency window of 0.10–1.00×10^5 Hz at 0.17 V were plotted (Fig. 2b). By comparing the curves related to GCE and GO/GCE, a relatively larger R_{ct} value related to the interface was obtained at the GO/GCE ($R_{ct} = 104.00$ Ω and $R_{ct} = 1020.00$ Ω). It may be attributed to the electrostatic repulsion between the -COO$^-$

groups on the GO sheets onto the electrode surface and the redox probe. Subsequently, by electrodeposition of the rGO onto the activated GO/GCE surface, a very much lower resistance was observed ($R_{ct} = 260.00$ Ω). This lower value may attribute to the good conductivity of the electrodeposited rGO. By coating of the β-CD film onto the rGO/GO/GCE surface, the efficient nanocomposite was synthesized onto the electrode surface. So, the R_{ct} value decreased to 375.00 Ω. The

equivalent circuit is shown in the inset. This nanocomposite suggests that the β-CD/rGO onto the GCE surface can accelerate electron transfer and increase the electrode surface area. The herein reported EIS data corroborate the preceding results obtained by CV and both of them confirm that the sensing interface is successfully achieved.

To study the morphology of the modified electrodes, SEM technique was applied (Fig. 2c). The bare GCE with an amorphous carbon morphology is indicated in Fig. 2c, a. As can be seen in Fig. 2c, b, the rGO/GCE surface shows a wrinkled graphene morphology. This morphology may attribute to this fact that the rGO is not capable of returning to a graphitic structure. The structure change in Fig. 2c, c indicates electropolymerizing of the β-CD onto the rGO/GCE surface is successfully achieved. The images corroborated that the synthesis of nanocomposite with a nanosize structure on the electrode surface is successfully performed. This strategy can serve as a good way to increase the surface area of the modified GCE.

In order to investigate the surface area of the modified electrode, active surface area value (A) was studied. To do so, the CVs plots in the electrolyte solution at different scan rates (v) ranging from 0.01 V s^{-1} to 0.10 V s^{-1} was recorded. According to Randles–Sevcik equation [25], the calculated A value of the β-CD/rGO/GCE was estimated to be 1.50 (cm^2) in comparison with the surface of the bare GCE, 0.066 (cm^2). According to these funding, utilizing the nanocomposite lead to an increase in the surface area of the modified GCE. Thus, this efficient platform may show high supramolecular recognition capabilities with enhancing conductivity.

To further study of the characteristic of the modified electrode, the DPVs were recorded in a 0.05 mol L^{-1} PB (pH = 5.00) without or with 0.1 mmol L^{-1} LEV (Fig. 2S). By comparison the results, it can be concluded that the β-CD/rGO/GCE is able to oxidize the LEV on the electrode surface with high efficiency. So, the β-CD/rGO nanocomposite has specific properties in modifying process of the electrode for LEV measurement.

Optimization of analytical conditions

To obtain the satisfying results, some factors such as the optimal amount of the used GO in the nanocomposite, accumulation time, pH effect and scan rate were investigated. Respective data and figures are given in the Electronic Supporting Material. The following experimental conditions were found to give best results for (A) optimal rGO loading: 3.00 μL; (B) accumulation time: 50 s and (C) best sample pH value: 5. To study the kinetic parameters and electrochemical behavior of the modified electrode, the CV technique was applied. The β-CD/rGO/GCE were recorded in a 0.10 mol L^{-1} PB (pH = 5.0) containing 10 μmol L^{-1} LEV at different scan rates ranging from 40.00 to 450.00 mV s^{-1} (Fig. 3). The results in Fig. 3,a reveal a good linearity relationship of the peak current (ΔI_{pa}) against the square root of the scan rate ($v^{1/2}$). This relationship reflects the oxidation reaction of the LEV on the modified electrode surface is diffusion-controlled. The logarithmic plot of the anodic peak current (log ΔI_{pa}) vs. scan rate (log v) had a slope of 0.4303 for LEV (Fig. 3c), providing further evidence of this fact [26]. Also, the anodic peak potential (E_{pa}) moved to more positive values upon an increase in the scan rate. This behavior reflects the irreversibility of the process [26, 27]. According to these funding, the oxidation mechanism of the LEV may be postulated as shown in Scheme 3 [26, 27].

Fig. 2 a The recorded CVs and (b) Nyquist plots of different modified electrodes in 5 mmol L^{-1} [Fe(CN)$_6$]$^{3-/4-}$ and 0.1 mol L^{-1} KCl (1:1) as a redox probe. Inset: an equivalent circuit for fitting the plots from the Nyquist plots. c The recorded SEM images of the (a) bare GCE, (b) rGO/GCE and (c) β-CD/rGO/GCE
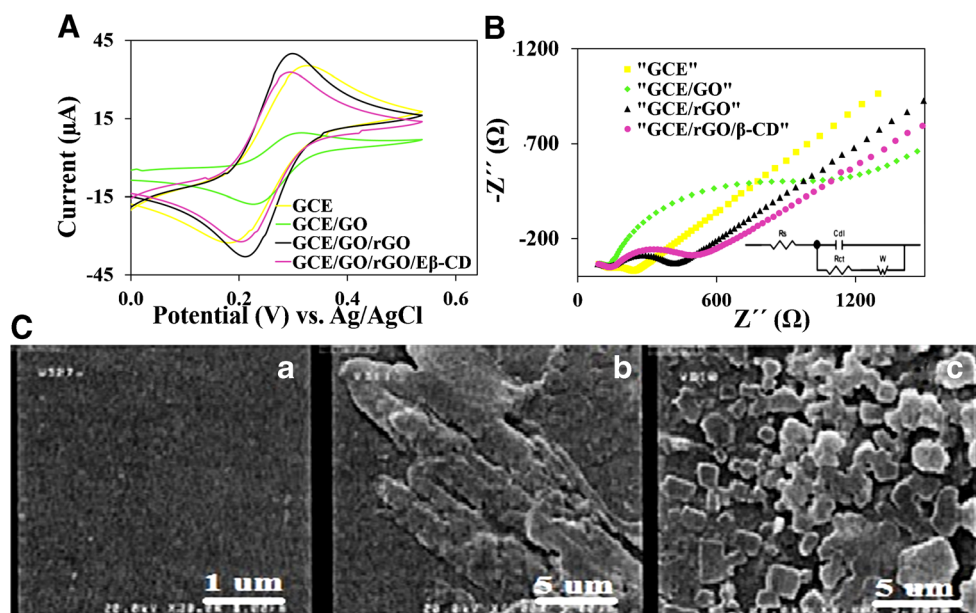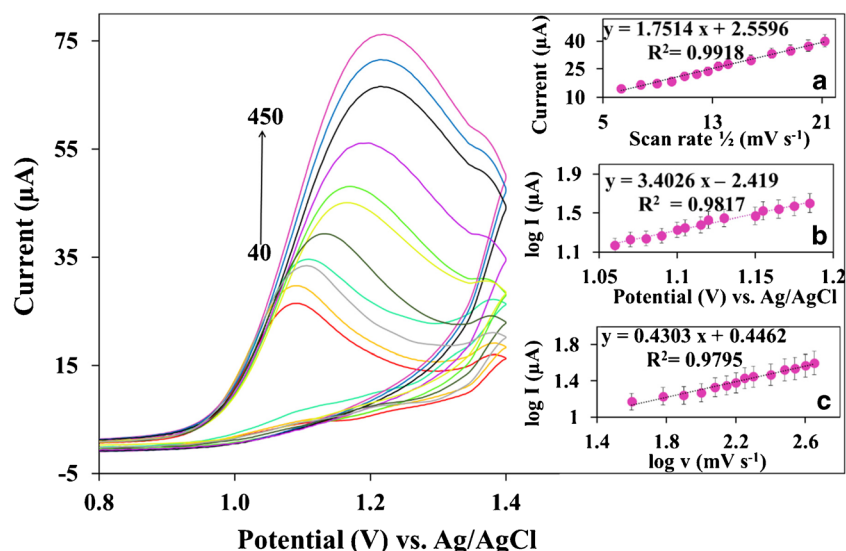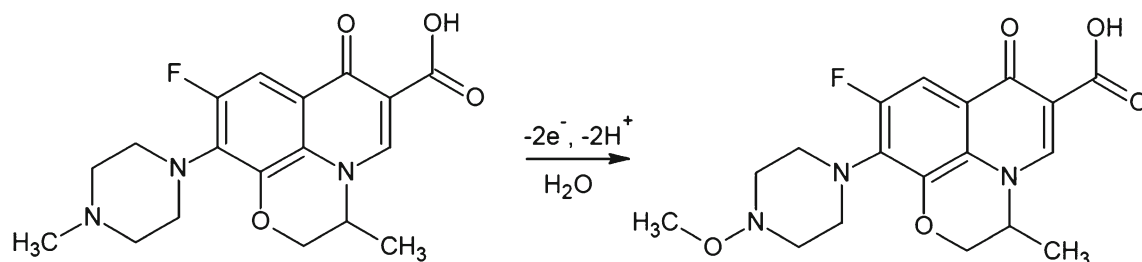
Fig. 3 The recorded CVs of the modified electrode in 0.10 mol L^{-1} PB (pH = 5.00) containing 10.00 μmol L^{-1} LEV at different scan rates ranging from 40.00 to 450.00 mV s^{-1} ((**a**) variation of I$_{pa}$ vs. $\nu^{1/2}$, (**b**) log Ipa vs. E value and (**d**) log I$_{pa}$ vs. log ν)

Evaluation of the analytical characteristics and applicability of the assay

To confirm the analytical applicability of the LEV sensor, the DPV technique was applied. A series of different concentrations of the target was measured in 0.1 mol L^{-1} PB (pH = 5.0) under the optimal conditions (Fig. 4a). As shown in Fig. 4a, a significant increase in the DPV signal is induced by increasing the LEV concentration. It can be referred to this fact that by adding more concentration of the LEV, more LEV can be oxidized on the nanocomposite surface in the sensor. The plots of I$_{pa}$ vs. LEV concentration were found to be linear in two ranges (inset a). A regression equation of I (μA) = 0.7010 log C (μM) + 3.77 (R^2 = 0.9933) was obtained for low concentration (100.00 pmol L^{-1} to 100.00 nmol L^{-1}). Also, a regression equation of I (μA) = 7.892 log C (μM) + 11.849 (R^2 = 0.9927) was estimated for high concentration (100 nmol L^{-1} to 100 μmol L^{-1}). The LOD (based on the signal to noise ratio of 3) and sensitivity values were calculated to be 30.00 pmol L^{-1} and 467.33 nA μM^{-1} cm^{-2}, respectively. Furthermore, the current density vs. the LEV concentration was calculated (inset b). The results reflect the method has a good sensitivity.

As presented in Table 1, the analytical characteristics of the sensor are compared with other methods in LEV detection.

Comparison of the results indicates that the strategy is comparable or even has better achievements in terms of determination, LOD and/or response linearity ranges. The sensor also offers many advantages including simplicity of the fabrication procedure without using toxic reagents, high stability, repeatability and reproducibility and low cost. The improved analytical characteristics of the sensor may be attributed to the good electrical conductivity as well as the presence of a high number of porous space in the β-CD/rGO nanocomposite. These results show the β-CD/rGO nanocomposite has an acceptable ability in preparation of an efficient platform in the LEV sensor.

Specificity, stability and repeatability of the assay

The anti-interference ability of the strategy regards to LEV detection in the presence of some inorganic ions and organic compounds as probable interferences were evaluated. The limit of tolerance was defined as the ratio of the activity of an interfering species to the LEV which led to a relative error of less than ±5.00%. 100.00 μmol L^{-1} of glucose, fructose and urea, 150.00 μmol L^{-1} of SO$_4^{2-}$, NO$_3^-$ and Na$^+$, 40.00 μmol L^{-1} of valine, 20.00 μmol L^{-1} of cysteine, 150.00 μmol L^{-1} of K$^+$, 15.00 μmol L^{-1} of ascorbic acid, 10.00 μmol L^{-1} of uric acid and dopamine was used.

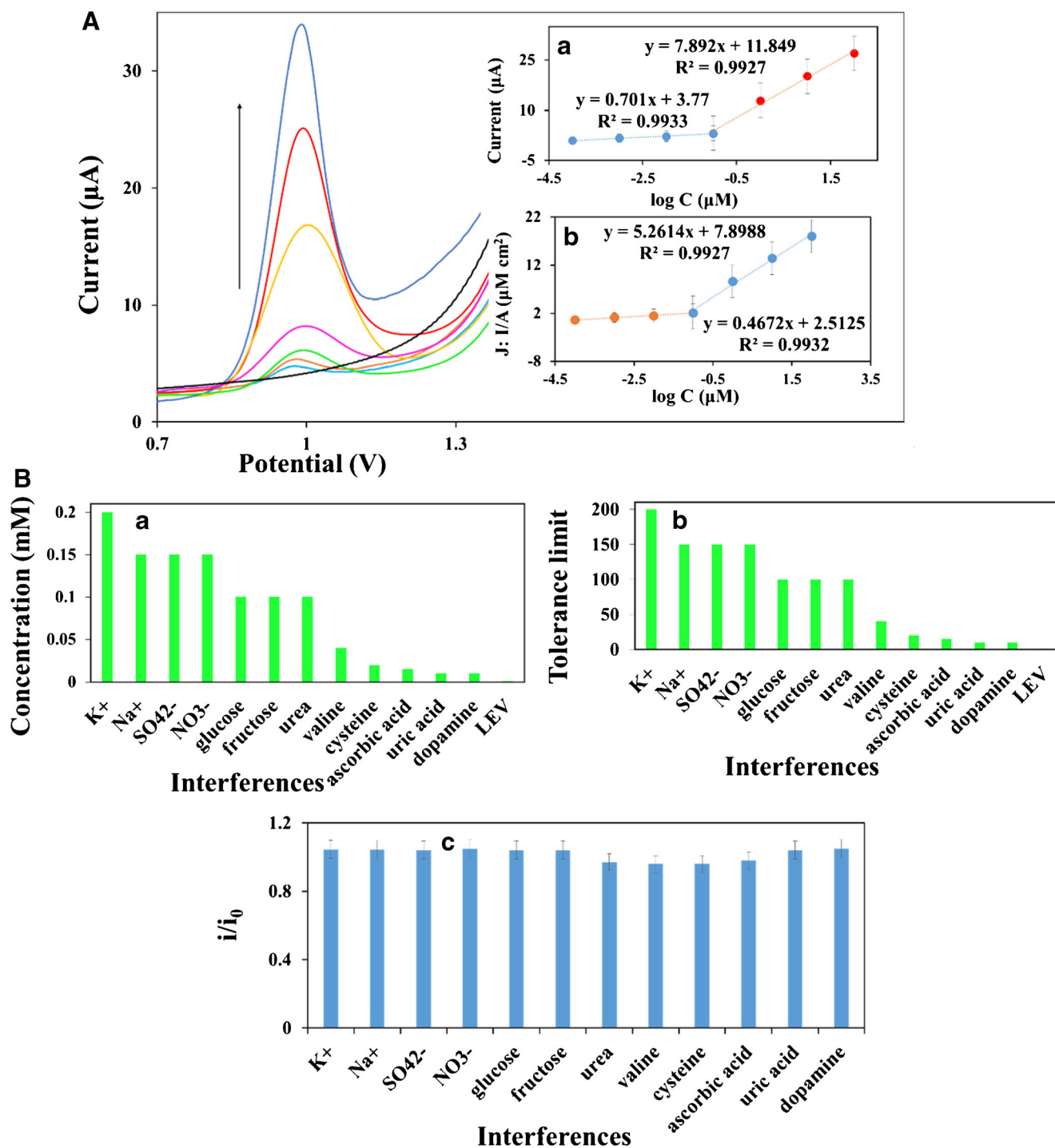**Scheme 3** The proposed reaction mechanism for oxidation of the LEV.

Fig. 4 **a** Histogram of the change of (**a**) concentration vs. different probable interferences (100.00 μmol L^{-1} of glucose, fructose and urea, 150.00 μmol L^{-1} of SO$_4^{2-}$, NO$_3^-$ and Na$^+$, 40.00 μmol L^{-1} of valine, 20.00 μmol L^{-1} of cysteine and 200.00 μmol L^{-1} of K$^+$, 15.00 μmol L^{-1} of ascorbic acid, 10.00 μmol L^{-1} of uric acid and dopamine) towards LEV (1 μmol L^{-1}), (**b**) tolerance limit vs. the interference and (**c**) current ratio vs.

the interference. **b** The recorded DPVs of the sensor in various LEV concentrations in two linear ranges. Inset a: the plot of I$_{pa}$ vs. LEV concentrations in the range of 0, 100.00 pmol L^{-1}, 1.00 nmol L^{-1}, 10.00 nmol L^{-1}, 100.00 nmol L^{-1}, 1 μmol L^{-1}, 10.00 μmol L^{-1}, and 100.00 μmol L^{-1}. Inset b: current density vs. the same LEV concentrations in inset a. Electrolyte: 0.10 mol L^{-1} PB (pH = 5.00)

According to the result, it was fund this substrate did not interfere with 1.00 μmol L^{-1} LEV detection according to the strategy (Fig. 4b). Histogram of the change of (a) concentration vs. different probable interferences towards LEV is shown

in inset a of the Fig. 4b. Also, Histogram change of the tolerance limit vs. the interference (inset b) and current ratio (i/i$_0$) vs. the interference (inset c) was investigated. Current ratio (i/i$_0$) means a ratio of current response of the LEV in presence

Table 1 Comparison of the performances of different electrochemical sensors for determination of levofloxacin

Electrode		Linear range (μmol L^{-1})	LOD (μmol L^{-1})	Ref.
MWCNTs[a]/poly(o-aminophenol)/GCE	DPV	3.0–200.0	1.0	[6]
PDDA[b]-rGO/AuNPs[c]/GCE	LSV[d]	10.0–200.0/200.0–800.0	3.9	[28]
MIP[e]/Gr[f]-AuNPs/GCE	DPV	1.0–100.0	0.53	[9]
dsDNA[g]/GCE	SWASV[h]	0.5–5.0	0.1	[29]
MWCNT-SnO$_2$/GCE	DPV	1.0–9.9	0.2	[7]
ssDNA[i]/SWCNT[j]/GE[k]	SWV[l]	1.0–10.0	7.52×10^{-2}	[21]
AgNPs[m]/CeO$_2$-Au/GCE	DPV	0.03-10	0.01	[13]
poly(p-ABSA)[n]-rGO/GCE	LSV	2-30/30–500	0.12	[14]
β-CD/rGO/GCE	DPV	1.0×10^{-4}–0.1/0.10–100.0	3.0×10^{-5}	This work

[a] Multi-walled carbon nanotubes

[b] Poly(diallyldimethylammonium chloride) solution

[c] Gold nanoparticles

[d] Linear sweep *voltammetry*

[e] Molecular imprinted polymers

[f] Graphene

[g] double-stranded DNA

[h] Square-wave anodic stripping voltammetry

[i] single-stranded DNA

[j] Single-walled carbon nanotubes

[k] Gold electrode

[l] Square-wave voltammetry

[m] Silver nanoparticles

[n] poly (p-aminobenzene sulfonic acid) (poly(p-ABSA))

of different interferences (i) toward the current response of the LEV (i$_0$). These acceptable results show the sensor based on the nanocomposite has unique properties that may be a good choice in the LEV detection in some samples with a complex matrix. To evaluate the stability of the method, the DPV signal of the sensor was recorded in the presence of 1.00 μmol L^{-1} LEV every four days during a 16-day period. It was found that after this period, the sensor produced 85.00% of its original current signal with a 15.00% relative standard deviation (RSD). This funding reflects the long lifetime of the sensor in LEV detection. The repeatability of the results was estimated by recording DPV signals in presence of 1.00 μmol L^{-1} LEV by using six β-CD/rGO/GCEs in the same manner. The

value of the RSD was estimated to be 2.00%. This value indicates the suitability of the β-CD/rGO nanocomposite for oxidizing of the LEV.

Analysis of the real sample

The applicability of the sensor in the analysis of the real sample was performed in some blood serum samples. The preparation of the real samples is described in the Experimental section. The DPV technique was used as the measuring technique and the standard addition method was adopted for this purpose. By adding the certain concentration of the LEV, the DPV signal was recorded. Finally, the obtained concentration

Table 2 Determination of the LEV in human blood serum samples by the sensor. (The means of three different determinations)

No.	Primary (μmol L^{-1})	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)
1	–	1×10^{-3}	0.96×10^{-3} (± 0.03)	96.00 (± 0.04)
2	–	5×10^{-3}	4.78×10^{-3} (± 0.02)	95.6 (± 0.05)
3	–	0.5	0.485 (± 0.03)	97.00 (± 0.04)
4	–	2.50	2.45 (± 0.04)	98.00 (± 0.03)
5	–	5.00	5.27 (± 0.02)	105.40 (± 0.04)
6	–	7.50	7.28 (± 0.03)	97.06 (± 0.28)

values and the calculated recovery value were compared by the spiked concentrations in the serum. The RSD value for three measuring was obtained (Table 2). The recovery values ranged from 95.60% to 105.40%. These results demonstrate the strategy has promising potential for LEV detection in the real sample.

Conclusions

A modified GCE was developed and used for LEV detection based on an efficient nanocomposite through an electrochemical method. The results show that the LEV sensor presents good stability, excellent ability and efficient applicability in measuring of the LEV. The strategy is simple and does not require any expensive materials, label, enzyme, redox indicator or even, complicated preparation method. Electrodeposition step is one of the easiest methods that is applied in this strategy. This acceptable funding relies upon some amplification features. (1) Utilizing rGO in the nanocomposite with relatively strong conducting and catalytic properties lead to an increase in the electron transfer rate. (2) Presence of the β-CD with unbeatable properties of incorporate molecules with appropriate size into its cavity may act as a molecularly imprinted polymer (MIP). (3) Electropolymerization of this substrate onto the rGO/GCE surface cause a specific cavity in selective detection of the target. (4) The clever combination of the β-CD and rGO to fabricate the nanocomposite can merge synergistic properties of them to prepare of a unique platform for efficient measuring of the LEV. Consequently, the strategy may make a promising candidate for the practical determination of the LEV in the real sample.

Compliance with ethical standards The author(s) declare that they have been complianced all ethical standards..

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