Levofloxacin enantioselective voltammetric sensing based on functionalized fullerene

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Abstract

There is a growing interest in the chiral analysis of antibacterial compounds, especially in the determination of trace amounts of an analyte in real samples. Unlike chiral chromatography and capillary electrophoresis, enantioselective voltammetric sensors can be easily adapted to analyze a wide range of substances and included in portable devices that allow screening of biological samples of complex composition. In this study the glassy carbon voltammetric sensors (GCE) based on layer-by-layer deposited graphene oxide (rGO) and functionalized fullerene (C60) were proposed for the levofloxacin (Lev: S-(–)-Ofloxacin) sensing. Since Lev is an optically active substance, it can be assumed that the sensitive layer of sensor with chiral selector will selectively interact with the antibiotic through various intermolecular interactions. Therefore, a number of selectors with different optical center configurations were obtained and studied, such as S/R-2-chloro-N-(1-phenylethyl)acetamide fullerene (S/R-C60AA), and S/R-N,N'-bis(1-phenylethyl)malonamide fullerene (S/R-C60MA).

Methodology

The proposed sensors were characterized by cyclic voltammetry (CV): over the range from −0.5 to +1.0 V with scan rate 0.1 Vs^{-1} and electrochemical impedance spectroscopy (EIS): the frequency range from 100 kHz to 0.1 Hz at the potential +0.24 V versus Ag/AgCl with an amplitude of 10 mV using standard redox probe of 5.0 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl. Differential pulse voltammetry (DPV) measurements of Lev were carried out over a potential range from +0.6 to +1.5 V with an amplitude of 0.075 V, a modulation time of 0.075 s and a scan rate of 30 mVs^{-1}.

Sensors manufacturing: 0.6 µl of GO in N,N-dimethylformamide (4 mg mL^{-1}) was dropped after ultrasonication on pre-polished by Al_2O_3 bare GCE. Electrochemical reduction was conducted in 0.05 M KH_2PO_4/Na_2HPO_4 with pH 6.86 by applying −0.8 V over 130 s. The modified GCE was placed under IR lamp until completely dry. Chiral selectors were dissolved in toluene (0.25 mg mL^{-1}) and dropped on the GCE/rGO surface in a volume of 1.5 µl, and then GCE/rGO/functionalized C60 was placed under IR lamp for 30 s.

Electrochemical and analytical performance of sensor GCE/rGO/S-C60AA for Lev determination

Primary analysis of the analyte and selector structures (Fig. 1) revealed possible binding sites such as aromatic moieties (π-π stacking) as well as electronegative and hydrogen atoms (hydrogen bonds).

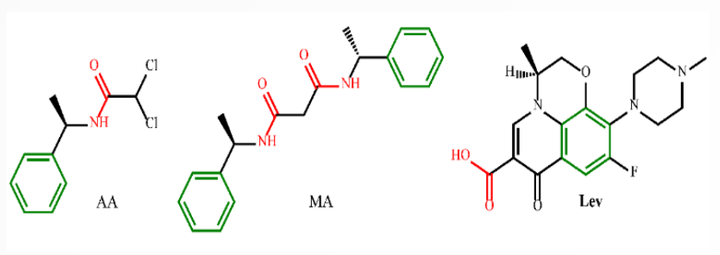

Fig. 1. Structure of selector and analyte molecules

It should be noted that the C60 structural block is a strong center for π–π stacking. Therefore, it should be further used as an independent interaction center or separated by a linker from the chiral center of the selector to ensure maximum selectivity and high sensitivity to the analyte. Computational studies showed that selectors with the S-configuration of the chiral center have the best binding to antibiotic. It should be noted that, in the case of C60AA derivatives, the difference in binding energies between the S- and R-selectors is greater (1.6 kcal mol^{-1}) than C60MA (0.9 kcal mol^{-1}).

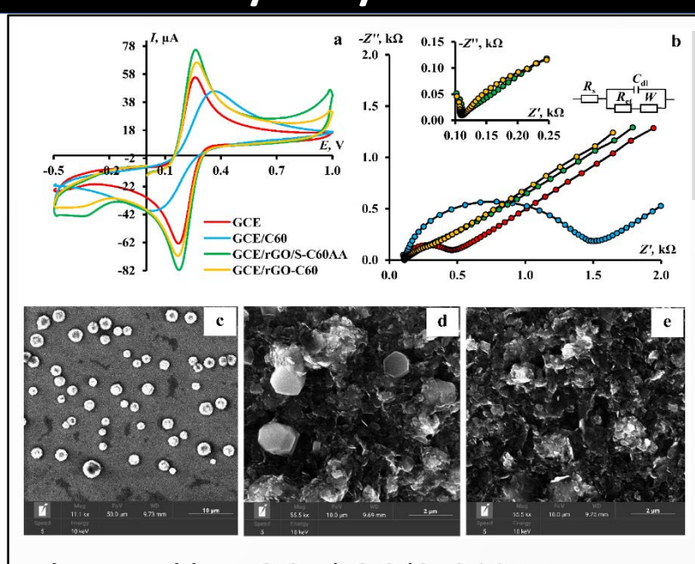

Fig. 2. (a) CVs and (b) Nyquist plots of 5 mM $[Fe(CN)_6]^{3-/4-}$ recorded using deferent electrodes. Inset b: Randles equivalent circuit. SEM images of (c) GCE/C60, (d) GCE/rGO/C60, (e) GCE/rGO/S-C60AA

Figure 2a shows CV responses with reversible redox peaks characterized by one-electron reaction at all electrodes. The most minimal CV response were observed on the sensor GCE/C60. Therefore, it was decided to use rGO as an electroactive substrate.

The resulting GCE/rGO/S-C60AA sensor showed the best CV response. In order to study the electron transfer resistance (Ret), Nyquist plots were plotted using different electrodes (Fig. 2b). The values of Ret decrease in the following sequence: GCE/C60 (Ret = 1.24 kΩ), unmodified GCE (Ret = 0.31 kΩ), GCE/rGO/C60 (Ret = 0.15 kΩ), GCE/rGO/S-C60AA (Ret = 0.10 kΩ). Thus, layer-by-layer deposition of functionalized C60 on the GCE/rGO significantly accelerates the process of electron transfer due to synergistic effect.

The highest analytical signal of Lev oxidation from CV (Fig. 3) is observed on the final sensor GCE/rGO/S-C60AA. The DPV revealed that the voltammetric determination of Lev is affected by the configuration of the chiral center of the selector, and an increase in the number of chiral centers in this case does not affect the outcome of determination. Sensors based on functionalized C60 with S-selectors in the sensitive layer showed the highest sensitivity to Lev (Fig. 4).

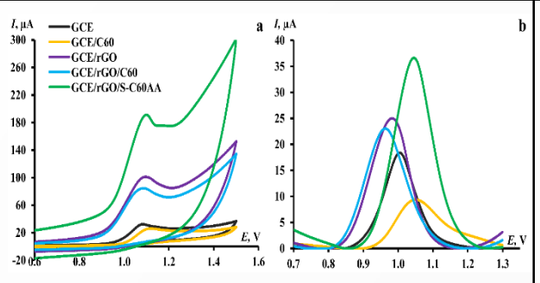

Fig. 3. (a) CV and (b) DPV responses of 0.5 mM Lev solutions recorded using deferent electrodes (pH 4.01; scan rate 0.1 Vs^{-1} according to CV, and 0.03 V s−1 according to DPV).

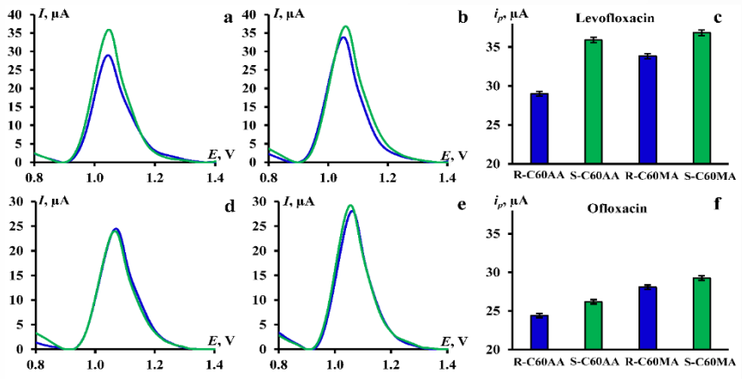

Fig. 4. DPVs of (a,b) 0.5 mM Lev and (d,e) Ofl solutions recorded using (a,d) GCE/rGO/C60AA and (b,e) GCE/rGO/C60MA. Histogram of the change of peak current of (c) Lev and (f) Ofl oxidation towards different configuration of chiral center of selector (pH 4.01; scan rate 0.03 V s^{-1}). The error bars reflect standard deviations from five measurements.

Lev was quantified using DPV. The sensor GCE/rGO/S-C60AA demonstrates two linear concentration ranges (Fig. 5). The sensor was successfully tested for Lev quantification in real samples of biological fluids and pharmaceutical formulations (Table 1).

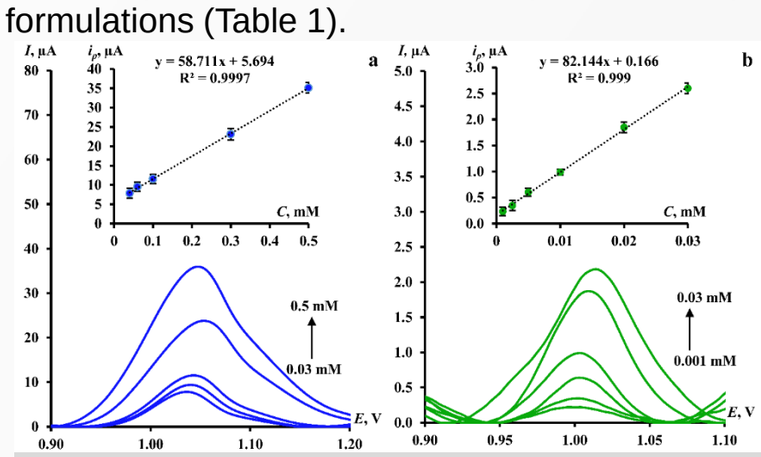

Fig. 5. DPVs of the sensor GCE/rGO/S-C60AA in various Lev concentrations in two linear ranges. Inset a: the plot of peak current vs. Lev concentrations in the range of 0.03, 0.04, 0.06, 0.1, 0.3, 0.5 mM. Inset b: the plot of peak current vs. Lev concentrations in the range of 0.001, 0.0025, 0.005, 0.01, 0.02, 0.03 (pH 4.01; scan rate 0.03 V s^{-1}). The error bars reflect standard deviations from five measurements

Table 1. Lev determination by spike-recovery test through DPV using GCE/rGO/S-C60AA (pH 4.01, scan rate 30 mVs^{-1}, n =5, P = 0.95)

Sample	Spiked, µM	Found, µM	RSD, %	Recovery, %
Human urine	15	15.2 ± 0.5	1.2	101.2
	35	35.8 ± 0.9	1.0	102.3
Human blood plasma	15	14.8 ± 0.3	0.8	98.7
	35	34.7 ± 0.6	0.7	99.2
Tablet	15	15.3 ± 0.5	0.4	102.1
	35	36.7 ± 1.8	1.7	104.4

Conclusions

The proposed sensor GCE/rGO/S-C60AA has good analytical and operational performance and surpasses some previously reports on Lev voltammetric sensing. Calibration plot was found to be linear in two ranges, such as $1.0 \cdot 10^{-6}$ – $3.0 \cdot 10^{-5}$ M and $3.0 \cdot 10^{-5}$ – $5.0 \cdot 10^{-4}$ M with sensitivity values of 82.14 and 58.71 µA/mM, respectively. The limits of detection and quantitation were calculated to be $3.7 \cdot 10^{-8}$ M and $1.2 \cdot 10^{-7}$ M, respectively. The developed sensor was successfully tested for pharmaceutical applications, in particular levofloxacin quantification in human urine, blood plasma and pharmaceutical formulations with the RSD ranged in 0.4 – 3.5 %, recovery values ranged in 91.5 – 104.4 %. Thus, fullerene C60 is mainly used as a reactive carbon matrix and its functionalization leads to the development of promising materials in sensing techniques for drug analysis.

Acknowledgements

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