Detection of Ofloxacin by Differential Pulse Voltammetry in Drugs Based on a Novel p-Aminobenzene Sulfonic Acid/Graphene Electrochemical Sensor

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This work demonstrates fabrication and performance (towards ofloxacin (OFL) detection) of a unique electrochemical sensor, composed of p-aminobenzene sulfonic acid (ASBA) and graphene (GR). For this purpose, a glassy carbon electrode (GCE) was coated by GR using electrochemical deposition and then electro-polymerized by ABSA. The surface and morphology of the resulting composite pASBA/GR/GCE were analyzed using scanning electron microscopy. Oxidation peak currents obtained from the differential pulse voltammograms with the assistance of the composite pASBA/GR/GCE showed linear correlation to the OFL concentrations in the 0.1–4.0 μmol/L range with the detection limits of acetaminophen is 0.03 μmol/L (S/N = 3). We also demonstrated OFL detection using pASBA/GR/GCE in pharmaceutical formulations. These results indicate that pASBA/GR/GCE has very strong potential as an electro-analytical sensor for the detection of components in pharmaceutical formulations.

Keywords: Differential Pulse Voltammetry Determination; Modified electrode; Graphene; p-aminobenzene sulfonic acid; Ofloxacin

1. INTRODUCTION

Electrodes modified using polymers are very popular in the electrochemical analysis because of their high sensitivity and accuracy as well as fast analysis speed, low cost, and easy preparation methods[1, 2]. Polymer-modified electrodes are widely used in biochemical and drug analyses to detect dopamine[3], uric acid[4], ascorbic acid[5] and acyclovir[6]. P-aminobenzene sulfonic acid (ABSA), as a conductive polymer, is especially suitable for the preparation of such electrodes as it
offers stable performance, good electrical conductivity, and high catalytic activity.

9-fluoro-2,3-dihydro-3-methyl-10-(4methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-
benzoazine-6-carboxylic acid, or ofloxacin (OFL), is a member of synthetic quinolones and is a
popular component against pathogenic gram-negative and-positive bacteria, which inhibited their DNA
gyrase. Besides OFL effectiveness, it is also inexpensive and can neutralize a wide variety of bacteria
as well as offers rapid treatment effect [7, 8]. OFL is widely used for both humans and animals for the
prevention and treatment of different infections and as a growth-promoting agent. However, synthetic
fluoroquinolones are often overprescribed, which is alarming because fluoroquinolones excess in the
body can cause complications during pregnancy and breastfeeding as well as harm babies, children,
and adolescents[9]. Therefore, governments of various countries implemented regulations on the
maximum residue levels of fluoroquinolones in a body after its consumption as well as withdrawal
periods[10]. The Ministry of Agriculture of the People's Republic of China announced in 2015 that
OFL is prohibited from being present in food for animals. Therefore, reliable and straightforward
method of detection of OFL and its residues in drugs and food is very critical.

Current techniques of OFL detection include spectrophotometry[11], high-performance liquid
chromatography[12,13], capillary electrophoresis[10,14] as well as electrochemical analysis[15,16]. In
this work, we focused on electrochemical detection of OFL using a novel electrochemical sensor based
on ASBA and grapheme (GR).

2. EXPERIMENTAL

2.1. Instrumental characterization

Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were
conducted using FEI Inspect F50 instrument at 10.0 kV. Prior to SEM, samples were covered with a
thin layer of gold. Electrochemical properties were tested using CHI 852C and 660D electrochemical
workstations fabricated by Chenhua Co. (China) using a standard three-electrode system containing a
glasy carbon electrode (GCE) 3.0 mm in diameter, saturated calomel electrode and a platinum
electrode, which acted as working, reference and counter electrodes, respectively. Differential pulse
voltammetry (DPV) was performed in 0.1 mol/L PBS solution (with pH = 5.0) containing 20 μmoL of
OFL at 0.1 V s⁻¹ scan rate. Cycle voltammetry (CV) was performed using 0.1 mo/L PBS solution (with
pH = 5.0) containing 50 μmol/L of OFL at different scan rates in the 0.02–0.28V s⁻¹ range.

2.2. Reagents

GR was purchased from Nanjing XFNANO Technology Co. OFL was obtained from Wuhan
Yuancheng Technology Co. Ear drops containing OFL are a commercial product of Shanghai HuangPu
YunJia Pharmaceutical Co. All other chemicals (which were of analytical grade) were purchased from
Sinopharm Chemical Reagent Co. Solutions were prepared using double-distilled water (DDW). 0.1
mol/L phosphate buffer solution (PBS) acted as a supporting electrolyte. All experiments and tests
were conducted at room temperature.
2.3 Sensor Preparation

GCE was glazed manually to a mirror-like surface using alumina slurry (with the particle size of ~0.5 mm), after which it was sonicated in HNO₃ (1:1), CH₃CH₂OH and DDW in an ultrasonic bath. After the electrode was thoroughly rinsed with DDW, it was placed in a 0.5 mol/L H₂SO₄ solution for activation, after which the electrode was dried in air at ambient conditions.

To modify the GCE electrode, 200 µL of 1 mg∙mL⁻¹ GR solution was added to 10 mL of 0.1 mol/L KCl solution and sonicated for 15 minutes until a homogeneous suspension formed. It was then used to cover the GCE electrode by electro-deposition at 1.7 V for 400 s. The resulting electrode was then immersed in 0.02 mol/L of pABSA for further immobilization, which was performed using cyclic voltammetry (CV) at in the -1.5–2.5 V range. The resulting composite pABSA/GR/GCE was carefully rinsed with DDW.

2.4. Sensor Performance Experiments

Interference experiments were conducted in 0.1 mol/L PBS solution (pH = 5.0) containing 20 µmol/L of OFL and certain interference substances: NaCl, KCl, glucose (Glu), glycine (Gly), alanine (Ala), phenylalanine (Phe), arginine (Arg), lysine (Lys), leucine (Leu) and citrate acid (CA). Concentrations of KCl and NaCl exceeded OFL concentration by 100 times, while levels of all other substances exceeded OFL concentration by 25 times.

To test the electrode relative to the real-life solutions, we used commercial ear drops. For this purpose, 50 µL of the ear drops were mixed with 10 mL of 0.1 M PBS (pH = 5.0). After the oxidation current was measured, 10 µL of 0.01 mol/L OFL was added to the ear drop solution, and the oxidation current (Ip) measurement was repeated. The resulting oxidation current value was then used to determine OFL concentration using preliminarily obtained calibration curve.

3. RESULTS AND DISCUSSION

3.1 Characterization of the pABSA-GR composites

SEM showed that ABSA grew homogeneously on the GR nano-films (Fig. 1). P-ABSA were homogeneous visible on the surface of GR. Numerous folds of the GR flakes provided sufficient surface area for ABSA to adhere and to grow uniformly (Fig. 1). EDS of pABSA-GR material showed uniform distribution of C, O as well as a strong S peak (Fig. 2), confirming the successful preparation of the pABSA-containing GR films.
3.2 Electrochemical performance of composite pABSA/GR/GCE

The responses of the electrodes tested in this work were tested using DPV. Unmodified GCE demonstrated an oxidation peak current ($I_p$) at 0.613 μA (Fig. 3A, curve a). $I_p$ values of ABSA- and GR-modified GCE as a response to OFL presence were higher: 1.334 and 0.992 μA, respectively (Fig. 3A, curve b, c). Thus, after modification with ABSA, the composite electrode had more active sites and, as a result, higher electro-catalytic activity towards OFL. pABSA/GR/GCE demonstrated the most intense response to the OFL presence: its corresponding $I_p$ value was 4.884 μA (Fig. 3A, curve d), which is eight times higher than that of unmodified GCE. Thus, pABSA/GR/GCE fabricated in this work had higher electro-catalytic activity towards OFL than pure and ABSA- and GR-modified GCE.

CV results showed that the oxidation and reduction currents during OFL detection were proportional to the scan rate (Fig. 4B-D), which for the scan rates in the 0.02–0.28V s$^{-1}$ range can be expressed as $I_p$ (μA) = 121.13υ (V s$^{-1}$) + 2.3319. The excellent correlation of this equation with the experimental results ($R^2 = 0.998$) suggests that reactions of OFL on the proposed electrode was controlled by the diffusion controlled process. Additionally, the oxidation peak potential ($E_p$) shifted to more positively while the scan rates adding gradually, which can be inferred that the electron transfer is irreversible[17].

$E_p$ values also showed a good linear correction ($R^2 = 0.994$) with the experimental data when plotted as a function of Log of the scan rate ($log\upsilon$), which can be expressed as $E_p(V) = 0.0478 log \upsilon +$
Therefore, according to the calculation[18], the possible reaction mechanism is as shown in Scheme 2, which agreed with previously reported results[19].

Figure 3. (A) DPV results of unmodified GCE (a), pABSA-modified GCE (b), GR-modified GCE (c), and pABSA/GR-modified GCE (d) under the presence of 20 µmol/L of OFL. (B) CV results of the pABSA/GR/GCE at 0.02–0.28 V s^{-1} scan rates. CV curves were recorded in 0.1 mol/L PBS (with pH = 5.0) with the addition of 50 µmol/L of OFL solution. (C) The linear relationship of Ip vs V; (D) The linear relationship of log υ vs E_p.

Scheme 2. The oxidation mechanisms of OFL.

3.3 Effect of pH and ABSA on OFL detection

To explore the conditions for the best OFL detection, we used DVP to determine how pH of the solution affected OFL detection under the presence of pABSA/GR/GCE (Fig. 4A). DVP oxidation peak current increased as pH increased and reached its maximum value at pH = 5.0. At pH > 5.0, Ip decreased significantly. Therefore, pH = 5.0 was the best pH for OFL detection.
The number of electro-polymerization cycles also affected the electrochemical performance of the pABSA-modified GCE during detection of 20 μmol/L OFL solution (Fig. 4B). The best catalytic activity was observed for the pABSA/GR/GCE modified using pABSA obtained using 20 polymerization cycles. Electrode containing pABSA obtained using a higher number of polymerization cycles had significantly lower activity very likely because pABSA membrane was too thick for efficient electron transfer between OFL and the electrode. Therefore, electrode containing pABSA polymerized with 20 cycles was chosen for further tests as the best performing one.

![Figure 4](image)

**Figure 4.** Effect of pH (A) and (B) ABSA electro-polymerization cycles on the electrode oxidation current response to 20 μmol/L of OFL.

### 3.4 Calibration Curve

OFL calibration curves were obtained using optimized conditions discussed above. Ip increased as OFL concentration in the solution increased (Fig. 5). The linear regression equation for the 0.1–40 μmol/L OFL concentration range can be expressed as \( I_p = 0.4018 + 0.2425c \) (μmol/L). The correlation coefficient \( R^2 \) of this regression was equal to 0.996. The detection limit (S/N = 3) was 0.03 μmol/L. Thus, pASBA/GR/GCE had an excellent electrochemical performance for the determination of OFL in the 0.1–40 μmol/L range.

![Figure 5](image)

**Figure 5.** (A) DPV of pABSA/GR/GCE at different concentrations of OFL. (B) Calibration curve for OFL determination, the inset is a DPVs with a low concentration of APAP (0.1–10 μmol/L).
Table 1 compares the electro-catalytic performance of various electrodes towards OFL detection. The composite electrode fabricated in this work demonstrated performance comparable to other electrodes reported in the literature.

**Table 1.** Electrochemical performance of various electrodes relative to OFL detection

<table>
<thead>
<tr>
<th>modified electrodes</th>
<th>Linear Range (μmol/L)</th>
<th>Limit of Detection (μmol/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu$_2$O/Nafion/GCE</td>
<td>0.5–27.5, 27.5–280</td>
<td>0.34</td>
<td>[19]</td>
</tr>
<tr>
<td>HPMaFP/Ppy/GCE</td>
<td>2–10</td>
<td>0.065</td>
<td>[20]</td>
</tr>
<tr>
<td>PLS film/GCE</td>
<td>10–100</td>
<td>0.16</td>
<td>[21]</td>
</tr>
<tr>
<td>ZnO/GR/GCE</td>
<td>1–100</td>
<td>0.33</td>
<td>[15]</td>
</tr>
<tr>
<td>OLA-Fe$_3$O$_4$/MWCNTs/GCE</td>
<td>0.01–8.9, 11–41.8</td>
<td>0.06</td>
<td>[22]</td>
</tr>
<tr>
<td>TrpGO-CNT/GCE</td>
<td>0.01–100</td>
<td>0.001</td>
<td>[23]</td>
</tr>
<tr>
<td>pABSA/GR/GCE</td>
<td>0.1–40</td>
<td>0.03</td>
<td>This work</td>
</tr>
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</table>

3.5 Selectively, reproducibility and stability of the pABSA/GR/GCE

![Figure 6. Specificity of the pABSA/GR/GCE.](image-url)
Interference tests showed that even at concentrations exceeding OFL content by 100 (for KCl and NaCl) and 25 times (all other substances), NaCl, KCl, Glu, Gly, Ala, Phe, Arg, Lys, Leu and CA demonstrated no significant interference in OFL detection by the composite pABSA/GR/GCE (Fig. 6). Thus, the method developed in this work showed excellent selectivity towards OFL detection.

For stability experiments, used pABSA/GR/GCE was stored at 4 ºC for one week, after which OFL detection experiments were performed again. Ip decreased by only 3.98% in comparison to the Ip value prior to storage at 4 ºC.

### 3.6 OFL detection in real-life samples

The practical application of the method and electrode developed in this work was demonstrated by its application to OFL detection in commercial ear drops (Labeled 15 mg/5 mL). All the samples were determined five times under similar conditions, and the standard addition method was applied. Table 2 shows that our approach and our modified electrode were able to detect initial OFL concentration in the ear drops accurately as well as to detect additional amount of OFL added to the ear drop solution. Thus, composite pABSA/GR/GCE is a very promising system for electrochemical detection of OFL in real-life commercial solutions.

**Table 2.** OFL contents determined using pABSA/GR/GCE electrode in commercially available ear drops (n=5)

<table>
<thead>
<tr>
<th>NO.</th>
<th>Initial OFL concentration present in the ear drop solution (μM)</th>
<th>Initial OFL concentration present in the ear drop solution (mg/mL)</th>
<th>OFL added (μM)</th>
<th>OFL detected (μM)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.93</td>
<td>3.05</td>
<td>10.00</td>
<td>26.94</td>
<td>100.1</td>
<td></td>
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<tr>
<td>2</td>
<td>16.94</td>
<td>3.05</td>
<td>10.00</td>
<td>27.12</td>
<td>101.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>16.75</td>
<td>3.02</td>
<td>10.00</td>
<td>27.14</td>
<td>103.9</td>
<td>1.40</td>
</tr>
<tr>
<td>4</td>
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<td>3.08</td>
<td>10.00</td>
<td>27.26</td>
<td>101.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>17.06</td>
<td>3.07</td>
<td>10.00</td>
<td>27.17</td>
<td>101.1</td>
<td></td>
</tr>
</tbody>
</table>

### 4. CONCLUSIONS

This work reports a sensitive, stable, and easy-to-make electrochemical sensor for OFL detection. The electrode was composed of ABSA electro-polymerized on the surface of GR. High conductivity and high surface area of GR accelerated the electron transfer between OFL and the electrode surface while NH2 groups of ABSA formed hydrogen bonds with –OH of OFL, which increased sensor response to OFL. The excellent performance of this electrode system was because of the synergistic effect between ABSA and GR. We believe that this sensor shows great promise in detecting OFL in the real-life samples on industrial scale.
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