Simple Fabrication of Reduced Graphene Oxide – Ionic Liquid Composite Modified Electrode for Sensitive Detection of Sulfadiazine

Jing-Ying Ma¹, Xiao-Ping Hong², *

¹ Department of Construction, Zhejiang College of Construction, Hangzhou 311231, China
² Department of Chemistry, Zhejiang Sci-Tech University, Hangzhou 310028, China
*E-mail: xpnghong@sina.com

Received: 6 January 2020 / Accepted: 5 February 2020 / Published: 10 April 2020

A convenient amperometric sensor based on incorporation of the reduced graphene oxide (rGO) and N-octyl-pyridinium-hexafluorophosphate (OPPF₆) ionic liquid (IL) as the selective receptor was described. The synergistic cooperation of the rGO and OPPF₆ contributed to a perfect performance of the electrochemical sensor. Compared with a bare glassy carbon electrode (GCE), the composite of rGO-OPPF₆ greatly improved the performance for the detection of sulfadiazine (SD) including a low anodic potential, sensitive current response, and good selectivity. The trace residues of the SD could be linearly assessed in the concentration range of 0.22-63.00 μmol/L (μM) with a detection limit of 0.07 μM. Additionally, the prepared sensor was successfully applied for the detection of the SD in an animal feed with recovery higher than 90%. The results agreed well with those obtained by high-performance liquid chromatography.

Keywords: sulfadiazine, graphene, ionic liquid, food safety, sensor

1. INTRODUCTION

Sulfadiazine (SD), a typical member of sulfonamides’ class, is widely used as a veterinary antibiotic in the field of an animal feeding for prophylactic and therapeutic purpose. Overuse or improper application of the veterinary drugs during the animal feeding period will result in an occurrence of the excessive drug residues in animal food products such as sea food, fish, meat, milk, eggs, honey, etc. Finally, the drug residues will transfer into a human body through the food cycle and cause different health issues, such as liver injury, renal lesions, carcinogenic effect, and mutagenic action [1]. Accordingly, EU, USA, Japan’s, and China’s government have set strict limits for the content of antibiotics residues in animal food. Therefore, it is highly desired to perform a reliable detection of the SD residues in the foods of animal origin as well as in the animal feed. Traditionally, the methods, such
as high-performance liquid chromatography (HPLC) [2], mass spectrometry [3,4], fluorescence spectroscopy [5] and enzyme-linked immunosorbent assay (ELISA) [6] were successfully applied for detection of the sulfonamides’ residues. Although those classical methods are highly sensitive and very accurate, they also showed prominent disadvantages such as high cost, time-consuming, and professional technical skills [7]. Consequently, a simple, convenient, and sensitive methods for rapid determination of the sulfonamide’s residues in the animal derived food or animal feed is urgently needed. Noteworthy, electrochemical methods have shown a promising application in solving the above-mentioned challenges of traditional methods due to their convenience of low cost, superior sensitivity, and in situ measurement [8-10]. Pioneers in this field had developed various electrochemical devices for convenient and rapid determination of the sulfonamides’ residues [11-13].

To obtain promising performance, nanomaterials are used as a sensing receptor on the surface of normal electrode due to their unique chemical and mechanical properties [14,15]. Graphene, an artificial two-dimensional sheet of sp²-hybridied carbon atoms, has attracted a lot of attention of the scientists because of its qualities such as simple synthesis, low cost, and excellent electric conductivity [16-18], which are mainly due to the arrangement of the atoms in the form of sheets. This results in high interaction and carrier conductivity [19]. Hence, graphene or reduced graphene oxide (rGO) have been considered as an ideal sensing modifier for determination of the trace electroactive chemicals [20].

Recently, ionic liquids (ILs) has been proved to be an ideal medium for chemical reaction or promising supporting modifier of the electrochemical electrodes due to its inherited properties such as chemical inertness, safety, good chemical affinity toward organics, high electrical conductivity, wide electrochemical potential window, and good electrochemical stability [21-23]. Generally, the electroactive materials such as carbon black [24], carbon nanotubes [25,26], graphene [22,27,28], and other nanomaterials [28,29] were incorporated together with the ILs to form a composite sensor modifier for acquiring an enhanced performance of the sensors.

This study aims to develop a new strategy for efficient determination of the SD residues in the animal feed by applying the composite modified electrode fabricated with the rGO and N-octyl-pyridinium-hexafluorophosphate (OPPF₆) IL. The combination of nanomaterials and IL can enhance the individual characteristics of each compound resulting in the fast adsorption and ultrasensitive response toward the substrate molecules. The new modified electrode exhibits a strict limit of detection (LOD) for determination of the SD residue in the animal feed. Moreover, the repeatability, reproducibility, and storage stability of the modified electrode were also investigated and discussed.

2. EXPERIMENTAL PROCEDURES

2.1 Materials

Sulfadiazine (analytical grade) was purchased from Sigma-Aldrich (Shanghai, China). The OPPF₆ was obtained from Shanghai Chengjie Chemicals (Shanghai, China). Other chemicals were of analytical grade and were used as received without any additional purification. Double-distilled water was used in all experimental procedures.
The rGO materials were donated by colleagues and was produced by a two-steps strategy. Firstly, graphene oxide (GO) was prepared from the graphite flakes by following a previously described method [30]. Then, the GO was reduced by hydrazine to obtain the rGO materials according to the method described in the literature [31].

2.2 Apparatus

A CHI660B (Chenhua Instrument, Shanghai, China) electroanalytical workstation equipped with a three-electrode system was used for the electrochemical characterization. A glassy carbon electrode (GCE) or a modified electrode was used as the working electrode along with a platinum wire electrode as the auxiliary electrode and an AgCl/Ag electrode (support electrolyte: saturated potassium chloride solution) as the reference electrode. The morphology of the rGO was investigated by a field-emission scanning electron microscope (FE-SEM) (Hitachi S-4800, Japan).

A high-performance chromatography (HPLC) system (Shimadzu, Japan) coupled with a UV detector was applied during the testing procedure of real samples. The separation was achieved on a C18 reversed-phase column (4.6 × 250 mm, 5 μm, Shimadzu, Japan) with 25% acetonitrile/distilled water solution as the eluent. The flow rate of the eluent was 1.0 mL/min. The detection wavelength of the UV detector was set to 270 nm.

2.3 Fabrication of modified electrode

Homogeneous electrode modifiers were prepared by dispersing 1.00 mg of the rGO or 1.00 mg of the rGO mixed with 9.00 mg of the OPPF₆ in 10 mL of DMF as a solvent and sonicated for 2 hours, respectively. The GCE electrodes were used as the basic electrode. Before usage, the GCE electrode was polished with 0.05 μm alumina slurry and then rinsed with double-distilled water several times. Finally, a mirror-like surface was prepared for the electrode decorating. Two microliters of prepared rGO or rGO-OPPF₆ suspension was casted onto the pre-cleaned GCE surface. After evaporating the solvent under an infrared lamp, the rGO or rGO-OPPF₆ modified electrodes were obtained and named as rGO-GCE or rGO-OPPF₆-GCE. Prior to the usage, the newly produced modified electrode was scanned successively in 0.10 mol/L (M) of sulfuric acid as an electrolyte by cyclic voltammetry for obtaining the stable electrochemical properties.

3. RESULTS AND DISCUSSION

3.1 Characterization of the modified electrode

An ITO substrate with a size of 0.50 × 0.50 cm was prepared for the SEM characterization of rGO by sonication in acetone and distilled water and drying in nitrogen atmosphere. Scanning electron microscopy (SEM) images were taken to characterize the morphology of the nanomaterials. As shown in Fig. 1, the ultra-thin lamellate sheets of the rGOS appeared to be smooth with a few wrinkles. The
accumulation of some sheets which appeared in the image is attributed to the non-polarity and strong surface energy of the rGO sheets.

Figure 1. The morphology of the rGO produced in the laboratory

The electrochemical response of the rGO-OPPF$_6$-GCE sensor toward the SD was carried out by a cyclic voltammetry scanning technique. During the procedure, the 1.0 mM SD solution with 0.1 M sulfuric acid as the support electrolyte was prepared as a stock solution. The GCE, rGO-GCE or rGO-OPPF$_6$-GCE were used as the working electrode with a saturated AgCl/Ag as the reference electrode, and a platinum wire electrode as the counter electrode. The testing procedure involved two steps. Firstly, the working electrode was immersed into the SD stock solution for a certain amount of time allowing accumulation of the SD’s trace by physical adsorption or extraction depending on the forms of working electrodes. Secondly, the GCE, rGO-GCE or rGO-OPPF$_6$-GCE were scanned in the stock solution by cyclic voltammetry method (scanning potential: 0.65-1.20 V, scanning rate: 0.05 V/s), respectively. As shown in Fig. 2, a weak anodic wave was observed at 1.10 V with the GCE electrode as the working electrode (pink curve d), which was a typical electrochemical response corresponding to the oxidation of the phenylamino group of sulfadiazine molecule [32]. Subsequently, when rGO-GCE was scanned, the anodic wave of SD was enlarged obviously (blue curve b) with the slightly negative shift of anodic potential to 1.05 V. This interesting performance of the rGO-GCE was probably contributed to the electrochemical catalytic effect and the promotion of active electrode surface area after the rGO decoration on the GCE electrode, which enabled more anodic reaction of SD molecules on the electrode surface [15]. Surprisingly, a striking anodic current peak, which was enormously enhanced comparing to that of the rGO-GCE, was obtained at potential of 0.90 V when the rGO-OPPF$_6$-GCE was used as the working electrode (red curve a). This exciting phenomenon probably occurred due to the synergistic effect of the composite materials fixed on the surface of the sensor. The rGO enormously increased the active surface area of the electrode, while the OPPF$_6$ decorated on the surface of sensor significantly improved the enrichment performance of the SD onto the surface of the sensor. These results
demonstrated that the rGO-OPPF6-GCE electrode is sensitive and promising for analysis of the SD at the trace levels.

![Figure 2. Cyclic voltammetry response of the SD obtained on the rGO-OPPF6-GCE (curve a, c), rGO-GCE (curve b), and GCE (curve d) in the presence (a, b, d) and absence (c) of 1.0 mM of SD as the probe.](image)

3.2 Optimization of detection procedure

Generally, the influence of support electrolyte toward the cyclic voltammetry response of the SD on the rGO-OPPF6-GC electrode was assessed. During the procedure, 0.1 M sulfuric acid, 0.2 M hydrochloric acid, Britton-Robinson buffer solution (containing 0.04 M phosphoric acid, acetic acid and boric acid), sodium acetate – acetic acid buffer solution (0.2 M sodium acetate and 0.2 M acetic acid mixed in different ratios to obtain the solutions with the various pH values) were used as the supporting electrolyte with the pH value ranged from 0.7 to 5.6, respectively. As shown in Fig. 3(A), the potential of the anodic peak (Ep) of 1.0 mM SD solution was pH dependent. When the pH value increased, the anodic peak of the SD was consecutively lowered and negatively shifted. This phenomenon indicates that anodic reaction of the SD is proton dependent, which is in agreement with the reports of literatures [33, 34]. The linear relationship between the Ep and pH could be described by the equation 1 with a correlation coefficient \( r^2 \) of 0.986. The slope value of \( d\text{Ep}/d\text{pH} \) (49.6 mV pH\(^{-1}\)) was close to the theoretical Nernstian value of -59.0 mV pH\(^{-1}\), which suggested that the number of protons and electrons involved in the anodic reaction of the SD was equal [35].

\[
\text{Ep (V)} = 0.9396 - 0.0496 \text{pH (1)}
\]

The pH value of supporting electrolyte showed a striking influence on the anodic current response of the SD. The cyclic voltammetry (CV) response of the SD was assessed by varying the
support electrolyte maintaining the same substrate concentration. Just as Fig. 3 (B) shows, it was found that the anodic current responses were increasing with the pH value of the supporting electrolytes decreasing, which was in agreement with the statement of Su’s work [36]. This result was probably attributed to the increasing of substrate solubility and the participation of protons during the anodic reaction of the SD. Hence, in the following testing procedure, 0.1 M sulfuric acid was used as the specified electrolyte.

The influence of electrode modifier’s dosage on the anodic response was also studied by the CV technique. Just as shown in Fig. 3(C), the anodic peak current was enormously magnified in the case of rGO-OPPF₆-GCE comparing to that of the normal GCE electrode without a decoration. The peak’s current tended to a plateau when the modifier volume exceeded 4 μL. Hence, the optimum modifier volume was set to 4 μL in the following procedures. The anodic peak’s current response against the accumulation time of SD was also assessed in 0.1 M sulfuric acid solution with the substrate concentration of 1.0 mM. As shown in Fig. 3 (D), the peak currents promoted sharply with the increasing in accumulation time and tended toward the passivation when the accumulation time exceeded 60 s. This interesting result was probably attributed to the extraction performance of the OPPF₆ ionic liquid anchored on graphene and on the electrode surface.

**Figure 3.** The influencing factors on the response of the sensor. (A. Correlation of anodic potential against the pH of supporting electrolyte; B. Anodic current response of the SD in electrolyte with different pH; C. Interference of modifier volume; D. Effect of the accumulation time during the assessment against the anodic current)
3.3 Analytical properties

Furthermore, the chronoamperometric technique (i-t) was employed for the quantitative analysis and for chasing a higher sensitivity. During the procedure, the rGO-OPPF6-GCE electrode was applied as the working electrode. Based on the properties illustrated in Fig. 2, the working potential of the sensor was set to 0.90 V which was defined as the anodic potential of the SD in 0.1 M sulfuric acid solution. As seen in Fig. 4, with the successive spiking with the different volume of SD stock solution into 5.0 mL of supporting electrolyte, the anodic current increased proportionally and then reached a plateau in less than 3 s. It is verified that the anodic current response linearly responded against the total substrate concentration of the system after each injection. Just as the inset plot of Fig. 4 shows, the anodic current was linearly proportional to the concentration of the SD substrate in concentration range from 0.22 to 63.00 μM with the linear fitting equation of $I (\mu A) = 0.002 + 0.029 (\mu M)$ (correlation coefficient: $r^2 = 0.995$). A limit of detection (LOD) was 0.07 μM according to the 3S/k [37] where the k was the slope ratio of the above-mentioned fitting equation and the S was the standard deviation of the mean value for the signals obtained in supporting electrolyte blank solution. Although the LOD of the rGO-OPPF6-GCE electrode was inferior comparing to that of the traditional HPLC method, the prepared sensor showed remarkable properties with the satisfactory value of LOD and wide linear response range among the electrochemical methods. In Table 1, the authors compared the analytical performance of literature reports and present work for the determination of sulfonamides. Although the Mohammad’s [38] and Hassani’s work [39] exhibited amazing LOD of 3.7×10⁻¹⁰ μM/L and 0.17 n g/L, respectively, the electrochemical impedance spectroscopy (EIS) method had the disadvantages of expensive instrument needing and difficulty in operation. In spite of the extremely sensitive LOD obtained by Chasta [40], the linear range of the present work is not inferior to his work. Compared with the other results obtained by traditional electrochemical methods of linear sweep voltammetry (LSV) [41, 42], square wave voltammetry (SWV) [8, 36, 40], differential pulse voltammetry (DPV) [11, 12, 43-46] and i-t technique [47], the LOD and linear range of this work is also remarkable.

![Figure 4](image-url)  
**Figure 4.** Chronoamperometric response (0.90 V) obtained with the rGO-OPPF6-GCE sensor after the successive injection of different volume of the SD stock solution. Inset: Calibration plot of the anodic current against the SD concentration in the system.
Table 1. Comparison of different methods for the determination of sulfonamides.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyte</th>
<th>LOD (μM)</th>
<th>Linear Rang (μM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>SD</td>
<td>0.02</td>
<td>0.4-12</td>
<td>Muhammet [2]</td>
</tr>
<tr>
<td>LSV(MIP/Ni(OH)2/NF)</td>
<td>SPy</td>
<td>0.4</td>
<td>0.6-1340</td>
<td>Liu [41]</td>
</tr>
<tr>
<td>LSV(GQD@Nafion/GCE)</td>
<td>SMZ</td>
<td>74.8 μg/L</td>
<td>-----</td>
<td>Gondim [42]</td>
</tr>
<tr>
<td>SWV(CNT/DAN/GCE)</td>
<td>SFA</td>
<td>0.11</td>
<td>5-1500</td>
<td>Yadav [8]</td>
</tr>
<tr>
<td>SWV(DAN/GCE)</td>
<td>SMZ</td>
<td>0.00005</td>
<td>0.5-150</td>
<td>Chasta [40]</td>
</tr>
<tr>
<td>SWV(SPCE)</td>
<td>SMZ</td>
<td>0.16</td>
<td>1.0-500</td>
<td>Su [36]</td>
</tr>
<tr>
<td>DPV(BiFE)</td>
<td>SD</td>
<td>2.1</td>
<td>3.2-20</td>
<td>Campestrini [43]</td>
</tr>
<tr>
<td>DPV(CNT/BA/SPCE)</td>
<td>SSZ</td>
<td>0.3</td>
<td>1.0-14</td>
<td>Sadeghi [44]</td>
</tr>
<tr>
<td>DPV(FeZnO/CPE)</td>
<td>SMX</td>
<td>0.03</td>
<td>2.0-160</td>
<td>Meshki [45]</td>
</tr>
<tr>
<td>DPV(AuNPs/Gr/GCE)</td>
<td>SAM</td>
<td>0.01</td>
<td>0.1-1000</td>
<td>He [11]</td>
</tr>
<tr>
<td>DPV(MIP/NiCo2O4/Gr)</td>
<td>SMZ</td>
<td>0.169 μg/L</td>
<td>0.2-1000 μg/L</td>
<td>Wei [46]</td>
</tr>
<tr>
<td>DPV(MIP/CNT@COF/GCE)</td>
<td>SMR</td>
<td>0.1</td>
<td>0.3-200</td>
<td>Sun [12]</td>
</tr>
<tr>
<td>EIS(AuNPs/Gr/PGE)</td>
<td>SDM</td>
<td>3.7×10^{-10}</td>
<td>1.0×10^{-2}-10</td>
<td>Mohammad [38]</td>
</tr>
<tr>
<td>EIS(MNP/Au)</td>
<td>SG</td>
<td>0.17 ng/L</td>
<td>0.1-1000 ng/L</td>
<td>Hassani [39]</td>
</tr>
<tr>
<td>i-t (MIP)</td>
<td>SMX</td>
<td>----</td>
<td>0.8-170</td>
<td>Turco [47]</td>
</tr>
<tr>
<td>i-t (rGO-OPPF6-GCE)</td>
<td>SD</td>
<td>0.07</td>
<td>0.22-63</td>
<td>present work</td>
</tr>
</tbody>
</table>


The repeatability of this sensor was checked by injecting the 20 μL of the SD stock solution into 5 mL of blank support electrolyte ten times. The current response of each injection was collected and the relative standard deviation (RSD) of 0.93% was obtained. Five rGO-OPPF6-GCE electrodes were prepared using the same procedure described in the section 2.3 and were applied one by one for chronoamperometric characterization maintaining the testing parameters unchanged. The reproducibility was expressed by the standard deviation, with the value of 2.3% calculated by the current responses obtained by using these five electrodes. The stability of the rGO-OPPF6-GCE electrode was assessed with the same electrode by monitoring the anodic current response of a solution with a constant concentration (4.0 μM) of the SD. After 10-days storage at the ambient condition, the anodic current response of the electrode remained 93.2% of its original response obtained at the first usage. The results presented in this section indicated that the rGO-OPPF6-GCE electrode had good repeatability, reproducibility, and stability.

Some reductive chemicals with lower anodic potential such as ascorbic acid (AA), uric acid (UC) and dopamine (DA), would interfere the detection of the SD. These chemicals would be oxidized simultaneously at the surface of the rGO-OPPF6-GCE electrode when the working potential was set at 0.90 V for determination of the SD. Thus, a strategy of the interference response subtraction was applied. The procedure was carried out in two steps. Firstly, an interference current (I) was recorded at the
potential of 0.60 V, where the current response was comprised of the blank response and coexisting chemicals with the anodic potential lesser than 0.60 V. Secondly, a total current (II) was monitored at 0.90 V in the same testing solution. The net current, calculated by subtracting the current (I) from the current (II), was contributed to the response of the existing SD in the testing solution. Other inert matters, such as NaCl, MgSO$_4$, NH$_4$Cl, etc., had negligible interference with the SD analysis (Fig. 5). In the Fig. 5, the current signals promoted step by step with the injection of the SD stock solution, while negligible responses were observed when 0.01 M solution of NaCl (a), MgSO$_4$ (b) and NH$_4$Cl (c) were injected into the testing system, respectively. This result proved that the sensor had satisfactory selectivity.

![Current vs Time Graph](image)

**Figure 5.** Interference response after the injection of some inert chemicals: a. NaCl, b. MgSO$_4$, c. NH$_4$Cl.

### 3.4 Test in real samples for SD residues determination

Practical application of the rGO-OPPF$_6$-GCE sensor was evaluated by applying it to monitor the SD remains in the animal feed. Five hundred grams of the mixed feed was donated by a fish farm near the author’s laboratory and then reduced to the 100 grams by quartering method. After grinding and screening through 40 mesh sieves completely, 2.0 grams of the feed powder was precisely weighted and transferred into a clean colorimetric tube. Immediately, 20 mL of 0.1 M sulfuric acid solution was added to the colorimetric tube for extraction of the SD. After 10 minutes of sonication, the liquid phase was separated by centrifugation.

**Table 2.** Recovery of the SD in the spiked samples obtained by rGO-OPPF$_6$-GCE sensor and HPLC analysis (n=3).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Spiked (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensor</td>
<td>HPLC</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>1.04</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>20.00</td>
<td>18.71</td>
<td>18.16</td>
</tr>
</tbody>
</table>
The supernatant was used for the chronoamperometric testing. Another 2.0 grams of the feed powder was spiked with a certain amount of the standard SD stock solution and stirred vigorously. Then, the subsequent analytical procedure was carried out according to the procedure applied for the real sample. The sample pretreatment procedure for HPLC analysis was different from that of the sensor. In brief, 2.0 grams of the feed powder sample spiked or non-spiked with the SD stock solution was extracted with 50 mL of acetonitrile for 30 min and then concentrated to 2 mL. After passing the concentrated extract through an alumina microcolumn for cleaning purpose, the container was evaporated to the dryness and then extracted thoroughly by adding 2 mL of the HPLC eluent mentioned in section 2.2. The extract solution was applied for the HPLC testing. The results of the analysis are listed in Table 1. Although the HPLC method showed superior LOD, recovery for the SD obtained by using the sensor were higher than 90% which was similar to that obtained by HPLC analysis. This result indicated that rGO-OPPF<sub>6</sub>-GCE sensor had good accuracy and was applicable for determination of the SD in the animal feed.

4. CONCLUSION

In the present study, the rGO-OPPF<sub>6</sub>-GCE sensor has been successfully developed and applied for determination of the SD residue in the animal feed samples. The combination of rGO nanomaterials and OPPF<sub>6</sub> super solvent contributed to the creation of a new sensor with exciting advantages of low production costs, simple handling, while keeping high stability, good selectivity, and accuracy toward the SD residues in the animal products. This approach could be easily extended to the determination of other sulfonamide residues, which would allow the new potential application of this sensor in the food safety control.

ACKNOWLEDGEMENT
This work was financially supported by Science and Technology Plan Project of Zhejiang Province (Grant No. 2020C03087).

References

10. F. A. Gutierrez, J. M. Gonzalez-Dominguez, A. Ansón-Casaos, J. Hernández-Ferrer, M. D. Rubianes,