A highly sensitive biosensor based on methacrylated graphene oxide-grafted polyaniline for ascorbic acid determination

1 Introduction

Biosensors have attracted much attention due to their unique properties such as simple procedure, easy production, fast response and cost efficiency [1–3]. Polyaniline (PANI) is a semi-flexible conducting polymer of the organic semiconductor family [4], which has attracted intensive interest as a result of remarkable features including superior conductivity [5], environmental stability [6], intriguing redox process [7] and inexpensive starting material [8]. In multidisciplinary areas, various applications for PANI have been reported such as biosensors, supercapacitors, biofuel cells, actuators, corrosion protection, membranes, solar cell devices, and rechargeable batteries [4,9–12]. Tunable properties, good processability (facile synthesis process), affordability, suitable electrochemical and environmental stability, strong bimolecular interactions and intriguing acid/base and doping/dedoping properties have made PANI a promising polymer among inherently conducting polymers [10].

Graphene, a single layer of carbon atoms with sp² chemical bonds, is the base for all nanoscale carbon materials such as fluorine bucky balls and carbon nanotubes (CNTs) [13–20]. Simple production procedures in comparison with other carbon nanomaterials and several properties, including zero band gap, high conductivity, flexibility, exceptional chemical stability, extremely wide porous structure, high specific surface area, high mobility of charge carriers and cost efficiency, make the graphene a promising nanomaterial for the
next era [21]. Graphene-based materials have been extensively used in biosensor applications due to their exceptional electrical, electrochemical and optical characteristics [22,23].

Nanocomposites have attracted much attention due to their exceptional properties [17,24–41]. Mechanical performance and electrical conductivity are the main characteristics that are developed with the combination of various materials into a nanocomposite [42–46]. Recently, conductive nanocomposites have captured the great interest in bioelectronics and biosensing fields [47]. Among them, graphene-grafted PANI nanocomposites are valuable owing to their excellent characteristics [48–50]. Combination of conducting polymers into a conductive nanostucture enhances the capacity, sensitivity, selectivity and electrical conductivity depending on the preparation methods and morphology. There are π-conjugated electrons in both graphene and PANI. These composites possessed several features including enhanced mechanical strength and excellent electrical conductivity [51]. Also, graphene-grafted PANI nanocomposites are utilized for preparing the electrode substrates. Recently, graphene-grafted PANI nanocomposites with excellent electrochemical characteristics and great conductivity have been utilized for numerous purposes such as biosensors, energy storage tools and electrochemical devices [6].

Ascorbic acid (AA), a reducing agent and successful antioxidant, plays some roles in preventing radical-induced ailments such as tumors and neurodegenerative [14,52]. The deficiency of AA can cause scrubbing, whereas its overdose can lead to stomach cramps and diarrhea [53]. The determination of AA levels is critical for diagnosis of food ingredients. There is a crucial necessity for determining AA level for healthcare and food quality/security due to the healthiness and industrial worth of AA and its low dose in biological and food samples [52].

Electrochemical methods have established rapid and low-cost performance as well as fast response with high selectivity, stability and sensitivity in determining some biomolecules and analytes [54]. Nanostructured composites such as palladium (Pd) nanowire-modified graphene [55], multiwall CNTs dispersed in polyhistidine [56], Fe₃O₄@gold (Au)-loaded graphene [57] and ZnO nanowire on hierarchical graphene [58] were reported for developing the sensitivity and selectivity of AA. Moreover, other nanocomposites including graphene-grafted PANI [52], graphene-supported platinum (Pt) nanoparticles [59], over-oxidized polypyrrole, PdNPs/Au [60] and 3D graphene foam CuO nanoflowers [61] have been exploited for determining AA.

Our group previously synthesized NFG/AgNPs/PANI for AA biosensing, which was more complex and expensive [52]. Moreover, we synthesized methacrylated graphene oxide (MeGO)/PANI nanocomposite and characterized it by physiochemical and electrochemical tests [6]. In this study, a simpler nanocomposite based on MeGO-grafted PANI is applied as electrochemical biosensor that has several benefits including cost efficiency, high sensitivity and good selectivity over AA determination. The linear range and detection limit of the sensing device are 8–5,000 and 2 µM, respectively. This platform shows the excellent stability over electroactive compounds.

2 Materials and methods

2.1 Chemicals

The potassium ferricyanide (K₃[Fe(CN)₆]), potassium permanganate (KMnO₄), sulfuric acid (H₂SO₄), potassium nitrate (KNO₃), sodium nitrate (NaNO₃) and graphite fine powder (spectroscopic grade, particle size ≤50 µm) were obtained from Merck. Aniline (99%), dimethylformamide, phosphate-buffered saline (PBS), N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride were purchased from Sigma.

2.2 Synthesis of MeGO

Graphite oxide was synthesized via the modified Hummer method [62]. Then, MeGO nanomaterials were prepared based on our previous studies [21,49].

2.3 MeGO-grafted FTO electrode

For preparing the amended electrode substrate, FTO glass plates (8 resistance) with the surface area of 0.25 cm² were provided and sequential ultrasonic cleaning was performed for 10 min in isopropanol, ethanol, acetone and deionized (DI) water. Under Ar gas flow, the FTO sheets were dried. Then, MeGO suspended in DI was deposited (20, 30 and 40 µL) on the FTO surface by the cast coating method, and it was
allowed to dry at 45°C, and the optimum volume was selected (40 µL).

### 2.4 Electropolymerization of aniline

Electropolymerization of PANI on the electrode surface was established according to our previous study [6]. Briefly, electrodepositing PANI on the MeGO-grafted FTO was initiated with 20 successive cyclic voltammograms (CVs) in a solution consisting of 0.03 M aniline monomer. Then, 0.5 M H₂SO₄ was applied on the electrode surface.

### 3 Results and discussion

Transmission electron microscopy (TEM) and field emission scanning electron microscopy (FESEM) tests were applied for investigating the morphology, topology and uniformity of the functionalized MeGO and MeGO/PANI nanocomposite. Figure 1a depicts the TEM image demonstrating a very thin layer of MeGO. The MeGO synthesized in this research was more transparent and uniform in comparison with previous works [63]. Based on previous results, the graphene nanosheets with excellent transparency had flake-like shapes and wrinkles and were more stable upon the exposure to the electron beam.

Figure 1b shows the microstructure of MeGO/PANI sample by the FESEM analysis. A porous structure was observed after electropolymerization of PANI on the MeGO surface. The forming of the pellet/flake-like microstructure and variations in the topography and morphology of the MeGO substrate corroborated the formation of PANI on the surface (Figure 1b). Clearly, the black regions and transparent edges were ascribed to PANI and MeGO nanosheets in MeGO/PANI nanocomposite, respectively. AA, an important factor for the synthesis and maintenance of collagen in tissue regeneration [64], was tested on our biosensor to assess its performance. CVs of the electrode modified with MeGO/PANI nanocomposites were conducted in the absence and the presence of AA (Figure 2). The results showed the highest catalytic effect for the AA solution.

To obtain the reaction mechanism of AA with the electrode surface, the changes in the oxidation peak of AA in the nanocomposite electrode were examined at

![Figure 1: The morphology and uniformity of the amended electrodes: (a) TEM and (b) FESEM images of the MeGO and pellet/flake-like MeGO/PANI, respectively.](image1)

![Figure 2: CVs of (a) MeGO/PANI in 0.02 M PBS without AA and (b) with 10 mM AA at the scan rate of 100 mV s⁻¹.](image2)
different scan rates. The increase of scan rates from 10 to 700 mV s\(^{-1}\) slightly changed the oxidation peak potentials (Figure 3). AA oxidation peak current versus scan rate \(\sqrt{v}\) with higher regression coefficient is linear. This indicates that the reaction mechanisms of AA with the electrode surface follow the diffusion mechanism. Moreover, the peak potential shifts to more positive potential with the increasing scan rate, which is another sign for the diffusion mechanism of AA on the electrode surface. Oxidation peak potential of AA catalysis was selected for chronoamperometry techniques to obtain the linear range of the sensor.

Successive aliquots of increasing concentrations of AA were tested on the sensor to obtain amperometric responses of the nanocomposite-modified electrodes. The electrode shows amperometric responses proportional to the AA concentration. The MeGO/PANI electrode demonstrates higher current (less uniform response) along with higher noise compared to MeGO electrode at the same concentrations of AA. This can be attributed to the active edges of graphene, which result in better interactions with AA. The nanocomposite electrode presents greater stability than MeGO electrode.

Figure 4a depicts the current increase in AA level from 8 to 5,000 \(\mu\)M in 0.02 M PBS (pH = 7.4). A linear trend is observed between the peak current and the AA level in Figure 4b (with a correlation of \(R^2 = 0.99\)). The detection limit of the amperometric responses was evaluated to be 2 \(\mu\)M (S/N = 5). Therefore, by addition of the AA aliquot (dropwise) to the PBS buffer, the current response (output) of the nanocomposite-based biosensor dramatically promotes to the AA redox reaction linearly with analyte biosensing enviable range. As given in Table 1, the electroanalytic and sensing features of the functionalized MeGO/PANI nanocomposite are meaningfully more than the AA biosensors from the previous reports. Some samples have low detection limit, while others show wide linear sensing range. In comparison with other studies, our sensing device shows very low detection limit and wide linear range. Therefore, this strategy for developing an analytical device could be established as a promising protocol to promote the sensing performance.
Table 1: The comparison of the linear range and detection limit of the present study with others

<table>
<thead>
<tr>
<th>Electrode materials</th>
<th>Detection limit (µM)</th>
<th>Linear range (µM)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANI/PPS/Gr</td>
<td>5</td>
<td>100–1,000</td>
<td>[65]</td>
</tr>
<tr>
<td>NG</td>
<td>2.2</td>
<td>5–1,300</td>
<td>[66]</td>
</tr>
<tr>
<td>CoPc–MWCNTs</td>
<td>1</td>
<td>10–2,600</td>
<td>[67]</td>
</tr>
<tr>
<td>AGCE/ASOD</td>
<td>2</td>
<td>5–400</td>
<td>[68]</td>
</tr>
<tr>
<td>PdNi/C</td>
<td>0.5</td>
<td>10–1,800</td>
<td>[69]</td>
</tr>
<tr>
<td>MWCNT/CCE</td>
<td>7.71</td>
<td>15–800</td>
<td>[70]</td>
</tr>
<tr>
<td>Pt–Au hybrid</td>
<td>103</td>
<td>103–165</td>
<td>[71]</td>
</tr>
<tr>
<td>Chitosan–graphene</td>
<td>50</td>
<td>50–1,200</td>
<td>[72]</td>
</tr>
<tr>
<td>OMC/Nafion</td>
<td>20</td>
<td>40–800</td>
<td>[73]</td>
</tr>
<tr>
<td>ZnO/RM</td>
<td>1.4</td>
<td>15–240</td>
<td>[74]</td>
</tr>
<tr>
<td>MBMOR/P</td>
<td>12.1</td>
<td>20–800</td>
<td>[75]</td>
</tr>
<tr>
<td>Pd/CNFs</td>
<td>15</td>
<td>50–4,000</td>
<td>[76]</td>
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<td>PMPy/Pd</td>
<td>1,000</td>
<td>50–1,000</td>
<td>[77]</td>
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<td>DB71</td>
<td>1</td>
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<td>[78]</td>
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<tr>
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<td>8</td>
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<td>[79]</td>
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<td>120</td>
<td>400–6,000</td>
<td>[80]</td>
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<tr>
<td>PdNPs–GO</td>
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<td>[81]</td>
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<tr>
<td>Pt/Au/GCE</td>
<td>—</td>
<td>24–384</td>
<td>[71]</td>
</tr>
<tr>
<td>NFG/AgNPs (1, 90 s)/PANI</td>
<td>8</td>
<td>10–5,460</td>
<td>[52]</td>
</tr>
<tr>
<td>NFG/AgNPs (10, 90 s)/PANI</td>
<td>50</td>
<td>50–11,460</td>
<td>[52]</td>
</tr>
<tr>
<td>MeGO/PANI</td>
<td>2</td>
<td>8–5,000</td>
<td>Present work</td>
</tr>
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</table>

The selectivity of the MeGO/PANI nanocomposite was evaluated in the presence of some interferences. The current responses of the interfering species were also analyzed at the modified electrode. The selectivity of the sensor was tested in PBS 0.02 M (pH = 7.4) with 10 mM interferences and 5 mM AA. As shown in Figure 5, a significant current response was observed for AA redox reaction, while interferences could not influence the current responses. In spite of the high concentrations of interferences, negligible changes were sensed in the sensing outputs, demonstrating the excellent selectivity of the present platform upon AA determination.

4 Conclusions

The functionalized MeGO/PANI nanocomposite demonstrated an excellent sensing activity over AA redox reaction. The linear sensing range and the detection limit of the sensing platform were dramatically more than most cases. Electroanalytical and biosensing results illustrated that the combination of MeGO as a 2D nanostructure and PANI as a familiar conducting polymer played a significant role in bioelectrochemical sensing applications. AA was scrutinized as a bioanalyte for quick and careful sensing of AA.

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