Two-dimensional functionalised methacrylated graphene oxide nanosheets as simple and inexpensive electrodes for biosensing applications

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Published in Micro & Nano Letters; Received on 22nd June 2018; Revised on 6th December 2018; Accepted on 3rd January 2019

Biosensors based on immobilised enzyme propose determination of analytes with excellent selectivity and sensitivity while their stability is still a challenge. Thus, promoting the selective, sensitive, rapid, inexpensive, and reliable strategies to fabricate new biosensors is a critical issue for the analyte determination. In this study, a novel functionalised graphene, methacrylated graphene oxide (MeGO), was utilised as an advanced biosensing device for non-enzyme determination of ascorbic acid (AA). The MeGO-based sensor was fabricated by a very simple coating approach on a glassy carbon electrode. The cyclic voltammogram of the new biosensor demonstrated a pair of well defined, reversible, and stable peaks for redox systems in a buffer solution. The exploited nanobiosensor depicted high catalytic bioactivity towards the oxidation of AA at a positive potential in the buffer solution. The biosensing characteristics of the developed nanobiosensor presented excellent sensitivity, low limit of detection, and wide linear range. To the best of this study's knowledge, this is the first research that uses MeGO in biosensing applications. Moreover, the sensing characteristics of the device are compared with other developments previously reported for non-enzyme AA sensors.

1. Introduction: Ascorbic acid (AA) is a natural organic complex with antioxidant properties and one of the nutritional requirements for humans and its deficiency leads to disorders. The most traditional diagnostic techniques for determining AA work through an enzymatic reaction. The enzyme-mediated approaches are still applicable due to their great selectivity and sensitivity. Nevertheless, the recent developments in non-enzymatic electrochemical sensors amended using the advanced materials including metal nanoparticles, conductive polymers, and carbon nanostructures have formulated them to be highly selective for AA detection [1].

Recently, graphene nanostructures have been considerably investigated in nanobiosensing devices [1–3]. Graphene oxide (GO) is one of the finest nanoscaled materials for fabricating the electrochemical sensors because of its favourable catalytic characteristics [4, 5]. It is significant that functionalised graphene nanosheets have a remarkably large specific surface area (2D structures), favourable solubility in water and large functional group-based oxygen entities including hydroxyl, epoxide, and carboxylic acid groups, suggesting that GO sheets have numerous excellent promising applications [6].

Functionalising the advanced materials and nanostructures such as GO is of great importance for biomedical applications that have been tried by many researchers [2, 3, 5]. Cha *et al.* [7] suggested a novel functionalisation method based on the reaction of GO and 3-(trimethoxysilyl) propyl methacrylate (TMSPM) that could produce methacrylated graphene oxide (MeGO) for amending the GO stability in a hydrogel nanocomposite with no sheets aggregation. Mamaghani *et al.* [5] recently reported the fast synthesis of MeGO as a graphene-functionalised nanostructure. MeGO/methacrylated gelation (GelMa) was carried out via the photocrosslinking approach that showed outstanding toughness and excellent mechanical characteristics, especially better resistance to failure in the optimal 3% range than GO because of the covalent bond established between MeGO and GelMa hydrogel in the composite [7]. Moreover, MeGO could proliferate fibroblast cells and had no toxicity according to an MTT assay [7, 8].

We have already synthesised MeGO, characterised its structural features and compared its characteristics with GO [5]. Since there is no research on MeGO-based nanostructures in biosensing applications, it will be a fascinating turf to scrutinise its sensing characteristics. Here, in the continuance of our findings on the biomedical applications and determination of analytes and biomolecules [9–13], the formulation of a novel and smart nanobiosensing device-based MeGO nanosheets is explored for an inexpensive, simple, sensitive, and fast AA determination. MeGO is utilised for the electrochemical oxidation of AA at the glassy carbon electrode (GCE)-modified MeGO to develop a new non-enzyme electrochemical procedure.

2. Materials and methods: All reagents and materials exploited to MeGO synthesis were of analytical grade purchased from Merck and were utilised with no further purification. Ammonium hydroxide was provided from Merck (Darmstadt, Germany). Electrochemical assays and sensing measurements were obtained in a standard three-electrode cell connected to an AUTOLAB system with PGSTAT302N boards (Eco Chemie, Utrecht, Netherlands). An Ag/AgCl electrode was applied as the reference electrode. The phosphate buffer solution (PBS, 0.1 M) was provided from disodium hydrogen phosphate (Na_2HPO_4) and phosphoric acid (H_3PO_4). All sensing experiments were performed in 0.1 M PBS at 25°C.

Functionalised MeGO nanosheets [1] were synthesised according to our previous research [5]. The non-enzyme electrode was assembled by dispersing 20 µl of the MeGO solution onto the GCE surface. Then, the electrode was placed at room temperature for 12 h to obtain the dried electrode. The provided MeGO coating on the GCE was treated by deionised water and PBS (0.1 M, pH 7.0) to eliminate any unbounded MeGO structures. The schematic representation of the sensor fabrication process is illustrated in Fig. 1. The GCE surface area was almost 3.01×10^{-6} m^2.

3. Results and discussion: Electrochemical features of the bare GCE and GCE-coated MeGO were performed by cyclic voltammogram (CV) and electrochemical impedance spectroscopy (EIS) presented in Fig. 2. CVs were carried out in 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and PBS 0.1 M, (pH 7.4) at 100 mV/s.

The explored CV outputs of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ at GCE surface were exposed in Fig. 2*a*. The redox behaviour of

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doi: 10.1049/mnl.2018.5320

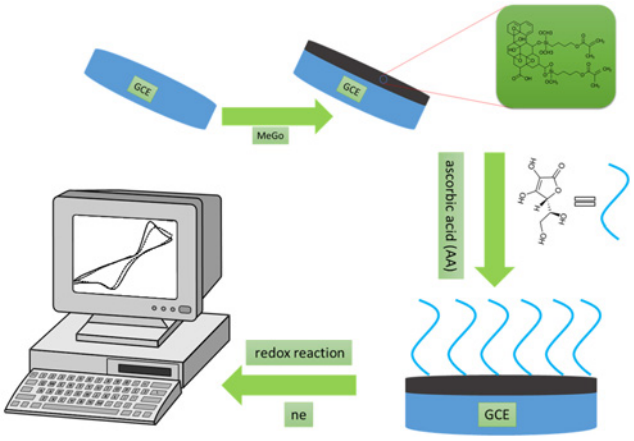
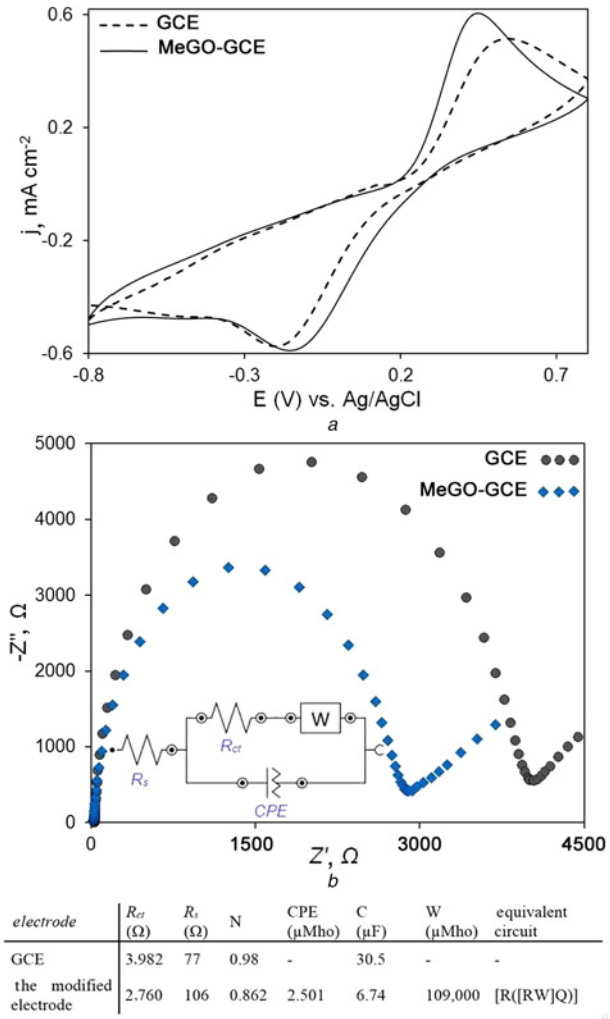


Fig. 1 *Schematic representation of modification of GCE for AA biosensor*



electrode	R_{ct} (Ω)	R_s (Ω)	N	CPE (μMho)	C (μF)	W (μMho)	equivalent circuit
GCE	3.982	77	0.98	-	30.5	-	-
the modified electrode	2.760	106	0.862	2.501	6.74	109,000	[R([RW]Q)]

Fig. 2 *GCE-modified MeGO in 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M PBS, at 100 mV/s (pH 7.4)*
a CVs
b EIS

$K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ showed a weak electrochemical property on the bare GCE with a large peak-to-peak potential separation ($\Delta E = 728$ mV) and expanded wave shape. The current response of the solution at the modified GCE enhanced and moved to 0.61 mA at +0.42 V and −0.59 mA at −0.17 V due to the appropriate conductivity of MeGO and its electrocatalytic bioactivity, which accepts redox electrons from the buffer and transports them to the GCE surface.

EIS is an effectual assay for determining the electron transfer features of the sensors and amended electrodes applied to understand the chemical transformations and events related to the conductive substrates [14]. Fig. 2*b* shows the Nyquist plots of the 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M PBS at the bare GCE and GCE-modified MeGO (pH 7.4). With introducing the MeGO to the GCE surface, the charge transfer resistance (R_{ct}) was measured to be lower than the bare GCE. It seems that the quicker kinetics of the redox reaction appeared on the GCE surface amended with MeGO in the presence of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ solution. Furthermore, MeGO in the modified layer has synergistic influences by enhancing the conductivity and active surface area.

Fig. 3 shows the increment in AA concentration from 20 to 4300 μM in 0.1 M PBS (pH 7.4), and a linear sensing relationship is observed between the peak current and AA concentration (with a correlation of $R^2 = 0.99$). The limit of detection (LOD) of the chronoamperometry assays was measured to be 10 μM ($S/N = 3$). Thus, by adding the analyte aliquot (dropwise) to the buffer solution, the current response of the nanosheet-based sensor to the analyte redox reaction enhanced linearly with AA sensing enviable range. The biosensor sensitivity was measured to be 2.9×10^{-3} A/(M cm^2). According to Table 1, the electrocatalytic

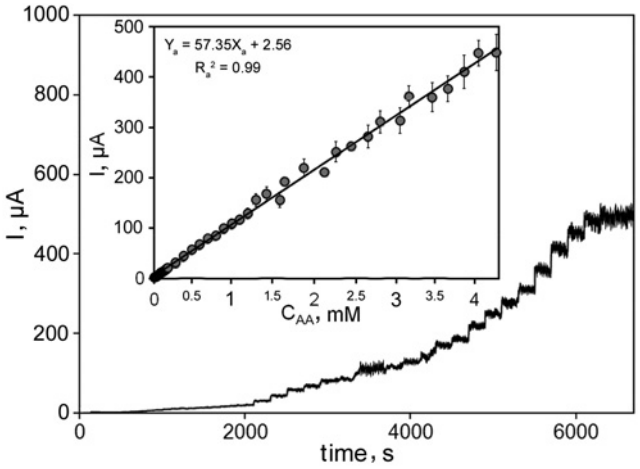


Fig. 3 *Amperometric responses and calibration curve (linear range) of sensor to AA redox reaction. Applied potential of amperometric detection was +0.42 V*

Table 1 Comparison of the LOD and linear sensing range of the present biosensor with other related research reported in the literature for determining AA

Electrode materials	LOD, μM	Linear range, μM	Reference
PANI/PSS/Gr	5	100–1000	[15]
NG	2.2	5–1300	[16]
CoPc–MWCNTs	1	10–2600	[17]
AGCE/ASOD	2	5–400	[18]
PdNi/C nanomaterials	0.5	10–1800	[19]
MBMOR/P	12.1	20–800	[20]
DB71	1	1–2000	[21]
$BPPF_6$/CPE	8	10–3000	[22]
PPF/GNS	120	400–6000	[23]
PdNPs–GO	—	20–2280	[24]
MeGO	10	20–4300	**present work**

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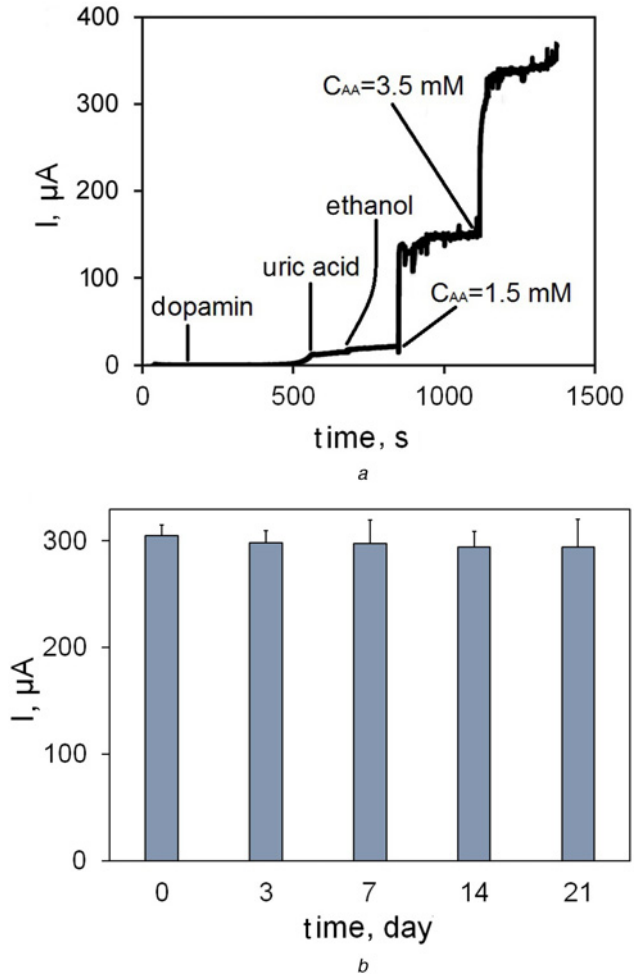

Fig. 4 *MeGO-modified electrode*
a Selectivity
b Stability

and biosensing characteristics of the functionalised graphene sensor were significantly more sensitive in comparison with some of those previously reported for AA sensors in the literature.

The output currents of the interferences were also tested at the MeGO electrode. The amperometric response of MeGO was evaluated in PBS 0.1 M (pH 7.4) with 10 mM interferents and 1.5 and 3.5 mM AA. A well-defined AA response was achieved (Fig. 4*a*) while no response was detected for the interfering species. The small responses for uric acid can be neglected. In other words, the MeGO-modified electrode exhibits excellent selectivity for AA detection.

The results of the electrode stability in Fig. 4*b* reveal that the current response retained >90% of its initial amount after 21 days.

4. Conclusion: The functionalised graphene, MeGO, synthesised in previous study showed an appropriate conductivity and excellent sensitivity. Electrochemical and sensing results provided from the experimental assays depicted that MeGO played as a promising nanostructure for electrochemical biosensing applications due to its excellent sensitive performance. AA was selected and scrutinised as a biological analyte for corroborating the assertion. The time-dependent chronoamperometry response of the GCE-modified MeGO nanosheets was interesting in a wide linear sensing range.

5. Acknowledgments: This study was funded by the Cancer Control Research Center, Cancer Control Foundation, Iran University of Medical Sciences, Tehran, Iran, as grant no. CCF-97052

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