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Voltammetric discrimination of mandelic acid enantiomers

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ABSTRACT

We report a novel electrochemical biosensor for direct discrimination of D- and L-mandelic acid (D- and L-MA) in aqueous medium. The glassy carbon electrode (GCE) surface was modified with reduced graphene oxide (rGO) and γ-globulin (GLOB). Electrochemical characterization of the modified electrodes was investigated by cyclic voltammetry and electrochemical impedance spectroscopy. The modified electrode surfaces were also characterized by scanning electron microscopy. Electrochemical response of the prepared electrode (GCE/rGO/GLOB) for discrimination of D- and L-MA enantiomers was investigated by cyclic voltammetry and was compared with bare GCE in the concentration range of 2 to 10 mM. Whereas the bare GCE showed no electrochemical response for the MA enantiomers, the GCE/rGO/GLOB electrode exhibited direct and selective discrimination with different oxidation potential values of 1.47 and 1.71 V and weak reduction peaks at potential values of −1.37 and −1.48 V, respectively. In addition, electrochemical performance of the modified electrode was investigated in mixed solution of D- and L-MA. The results show that the produced electrode can be used as electrochemical chiral biosensor for MA.

Chiral discrimination has become of increasing importance during the past decade due to the racemic switch strategy adapted by nearly all fields of biological, chemical, and pharmaceutical sciences [1]. This process generally includes the selective receptor that selectively forms complex with one of the enantiomers of a chiral molecule by noncovalent interaction such as hydrogen bonding, electrostatic interaction, or hydrophobic interaction. Such receptors not only provide a controlled means for studying the fundamentals of the interactions in nature but also open new ways to develop novel valuable enantioselective sensors, catalysts, and other molecular devices [2–4]. In the enantioselective sensors, electrochemical techniques have been attracted significant attention owing to some advantages over chromatographic and spectrophotometric techniques. In particular, the electrochemical technique offers the advantages of easy preparation and relatively low cost, and it requires only small sample volumes (microliter range) that couple with the very low detection limits. Due to the fact that relatively fewer electroactive interferents often come up than spectroscopic interferents, electrochemical detection in complex samples shows excellent results with high selectivity [5]. For these reasons, many efforts have been devoted to the goal of finding new modification materials and various organic redox mediators, nanoparticles, polymers, self-assembled monolayers, and carbon materials [6].

During recent years, as a new kind of carbon material, graphene has attracted enormous attention in constructing electrochemical sensors due to large specific surface area for the substances [7–11]. Because every atom in graphene is a surface atom, molecular interaction can be highly sensitive for absorbed molecules [12]. The electronic and mechanical properties of graphene can be changed by chemical modification to obtain graphene oxide (GO)[1] for use in graphene-based biosensors [13,14]. The presence of copious oxygen-containing groups on GO, such as alcohols, epoxides, and carboxyl groups, not only can significantly affect the van der Waals interactions between the graphene layers but also can render it strongly hydrophilic [15–17]. In addition, GO-based materials with tunable oxygen functionalities on their basal planes facilitate the surface modification with other materials [8,18]. However, GO is electrically insulating material; thus, it leads to significant losses of the superior properties of graphene [19]. In this respect, GO is generally reduced by reducing agents for the restoration of a graphitic network of sp^2-hybridized carbon. Reduced graphene oxide (rGO) is conductive and has chemically active functional sites, making it a frequently considered active material in molecular sensors [8,20].

The current study deals with a novel graphene oxide-based electrochemical biosensor based on γ-globulin (GLOB) for the

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[1] *Abbreviations used:* GO, graphene oxide; rGO, reduced graphene oxide; GLOB, γ-globulin; MA, mandelic acid; GCE, glassy carbon electrode; CV, cyclic voltammetry; SEM, scanning electron microscopy; EIS, electrochemical impedance spectroscopy; A–PBS, sodium acetate and phosphate-buffered saline; RSD, relative standard deviation.

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direct discrimination of mandelic acid (MA) enantiomers. Glassy carbon electrode (GCE) surface was modified with rGO and GLOB. The electrochemical behaviors of D- and L-MA at the GCE/rGO/GLOB surface were investigated by cyclic voltammetry (CV). The results show that the modified electrode not only can be used for determination of MA but also readily allows the direct enantiomeric discrimination of D- and L-MA in an aqueous medium. It also provides an opportunity to make successful electrochemical enantiomeric percentage analysis for MA.

Materials and methods

Chemical and apparatus

All chemicals were of analytical grade and used without further purification. Graphite powder (99.99 %), concentrated H_3PO_4 and H_2SO_4, H_2O_2 (30%), hydrazine hydrate solution, $KMnO_4$ (99%), D-MA, L-MA, and GLOB were purchased from Sigma–Aldrich. All aqueous solutions were freshly prepared using ultrapure water with a resistivity of 18.2 MΩ cm.

Electrode morphologies were investigated by scanning electron microscopy (SEM) performed on a Zeiss EVO LS 10 SEM at accelerating voltage of 20 kV and 5000× magnification. Before the scanning process, all samples were coated with gold to enhance the electron conductivity. Electrochemical measurements were performed with an Ivium CompactStat potentiostat (Ivium Technologies, The Netherlands) combined with a BAS/C3 electrochemical cell stand using a three-electrode configuration cell. GCE, Ag/AgCl electrode (with Luggin tip) in 3 M KCl, and platinum electrode were used as working electrode, reference electrode, and counter electrode, respectively. Electrochemical impedance spectroscopy (EIS) measurements were recorded in a mixed solution of 50 mM sodium acetate and 50 mM phosphate-buffered saline (A–PBS) containing 1.0 mM $[Fe(CN)_6]^{-3/-4}$ redox couples at pH 7.01. The impedance measurements were performed in a frequency range from 10 Hz to 100 kHz with a 5-mV signal amplitude. CV measurements were carried out in a mixed solution of A–PBS (pH 7.2 as an optimum condition) with 100 mM KCl at ambient temperature (25 °C). To avoid undesired effects, the electrochemical cell setup was freshly prepared and the measurements were performed by purging nitrogen gas for 20 min before each experiment. During the electrochemical experiments, nitrogen atmosphere was maintained over the solution.

Preparation of rGO

GO was synthesized from graphite powder by the improved method [21], which has significant advantages over Hummers and Offeman's method [22], with an additional preoxidation process. Briefly, 3.0 g of graphite was preoxidized in a mixture containing 1.5 g of $K_2S_2O_8$, 1.5 g of P_2O_5, and 15 ml of concentrated H_2SO_4. The mixture was then diluted with ultrapure water, filtered, and dried in a vacuum oven at 50 °C. After this process, the resultant graphite was reoxidized by the improved method containing $KMnO_4$ (18.0 g) in a 9:1 mixture of concentrated H_2SO_4/H_3PO_4 (360:40 ml) for production of GO. To prepare the rGO, 5 ml of GO aqueous suspension (0.5 mg/ml) was added into hydrazine hydrate (0.50 ml, 32.1 mM) in a round-bottom flask and was mixed in an oil bath at 100 °C for 24 h. The product was filtered and washed with ultrapure water (5 × 20 ml) and methanol (5 × 20 ml) and was dried in a vacuum oven at 50 °C [23].

Preparation of modified electrodes

The schematic diagram of a producing electrochemical biosensor for discrimination of MA enantiomers presented in Scheme 1 can be briefly explained as follows. Prior to each experiment, GCE was pre-cleaned with acetone, ethanol, and ultrapure water. Then the GCE surface was polished with 1.0, 0.3, and 0.05 μm alumina powder (PACE Technologies, USA) on a felt pad and was washed with a copious amount of ultrapure water. The GCE was then immersed in water and methanol for 15 min, respectively, in an ultrasonic bath (Sonorex Super RK 106, Germany) in order to remove residual alumina particles by sonication. The electrode was dried under N_2 atmosphere at room temperature before modification steps. After drying under N_2 atmosphere, a 5.0-μl suspension of rGO (0.5 mg/ml) sonicated for 1 h was dropped three times on the GCE surfaces (as an optimum condition) in order to obtain rGO-modified electrode (GCE/rGO). After rinsing with ultrapure water and drying under N_2 atmosphere, the electrode was immersed in a buffer solution containing GLOB (10 mg/ml, pH 7.2) for 40 h (as an optimum condition) at approximately +4 °C in order to prepare the GCE/rGO/GLOB.

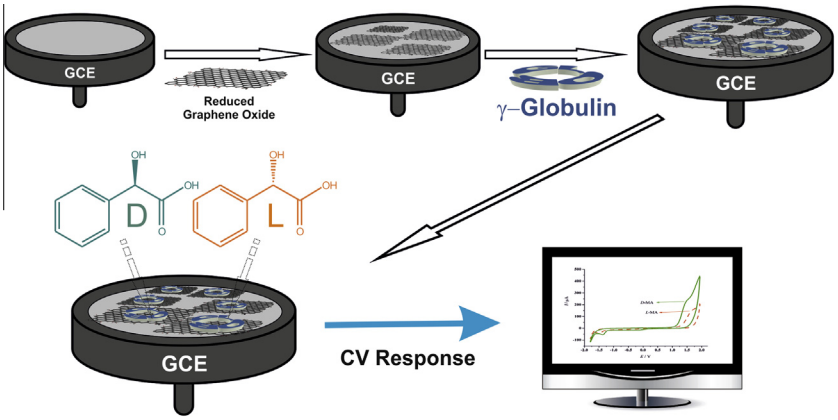
The GCE/rGO/GLOB electrode was then washed with ultrapure water and dried under N_2 before use. The electrode was stored in buffer solution at approximately +4 °C in a closed vessel when not in use.

Results and discussion

Surface and electrochemical characterization of modified electrodes

SEM was performed to characterize the surface morphology of the bare and modified GCEs at each step. Whereas the bare GCE has a smooth surface, as can be seen in Fig. 1A, the GCE/rGO surface shows that rGO nanosheets surround the electrode surface, as shown in Fig. 1B. The modification of GLOB on GCE/rGO electrode results in a well coverage of the GLOB molecules wrapped loosely by the sheets of graphene on the surface, as illustrated in Fig. 1C.

The characterization of the modified electrodes was also investigated electrochemically by CV and EIS in A–PBS solution (pH 7.01) containing 1.0 mM $[Fe(CN)_6]^{-3/-4}$ redox couples. The changes at each step of the surface modification of GCE can be easily seen by the peak-to-peak potential difference (ΔE_p) and the peak currents ratio ($I_{p,a}/I_{p,c}$) of the $[Fe(CN)_6]^{-3/-4}$ redox couple in Fig. 2A. The bare GCE shows a typical reversible electrochemical response for the $[Fe(CN)_6]^{-3/-4}$ redox couple at 224 mV with $I_{p,a}/I_{p,c}$ of 1.14 and ΔE_p of 112 mV [24]. The modification of GCE surface with rGO presents a decrease in $I_{p,a}/I_{p,c}$ of 0.81 and a substantial increase for ΔE_p of 281 mV. Considering the related studies, there are two main explanations for these changes. First, when the electrode is coated with GO derivatives, the peak currents decrease greatly, which can be attributed to the negatively charged carboxyl group on rGO edges blocking the diffusion of $[Fe(CN)_6]^{-3/-4}$ from solution to electrode surface [25,26]. Second, ΔE_P is inversely proportional to the electron transfer rate, so the results suggest that the film of GO derivatives acts as an insulating layer, resulting in difficult interfacial electron transfer due to their disrupted sp^2 bonding networks [26–28]. In a further step, GLOB modification on the GCE/rGO caused a significant change on the electrochemical behavior of the $[Fe(CN)_6]^{-3/-4}$ couple, leading to a significant decrease in $I_{p,a}/I_{p,c}$ of 0.43 and an increase in ΔE_p of 405 mV. This result was attributed to the hydrophobic or electrostatic/hydrogen bonding interactions between rGO and GLOB and gives rise to the blockage of the electrode surface for oxidation/reduction of the redox probe ($[Fe(CN)_6]^{-3/-4}$) [7]. EIS was used for confirming and deeply probing the interface properties of surface-modified electrodes. Fig. 2B shows the impedance responses (Nyquist plots) for the bare GCE (curve a), GCE/rGO (curve b), and GCE/rGO/GLOB (curve c) in the presence of 1 mM $[Fe(CN)_6]^{-3/-4}$ in A–PBS solution (pH 7.01). The diameter of the semicircle presents the charge transfer resistance



Scheme 1. Schematic representation of the discrimination of mandelic acid at the modified GCE based on rGO/GLOB.

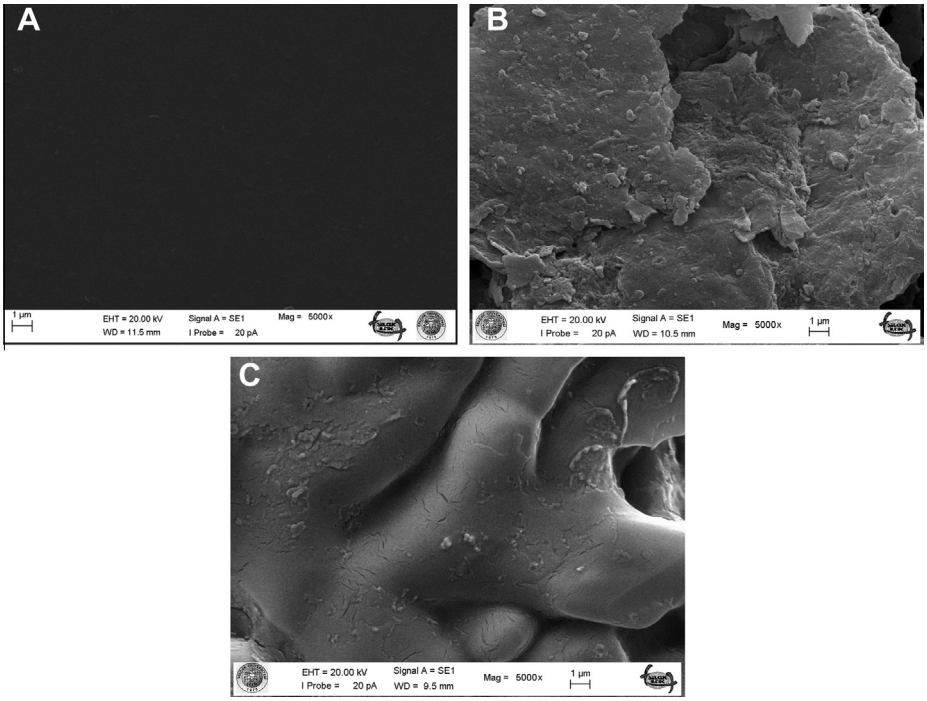


Fig.1. SEM micrographs of GCE (A), GCE/rGO (B), and GCE/rGO/GLOB (C).

that can be used to describe the interfacial properties of the modified electrode. The semicircular portion at higher frequency corresponds to the electron transfer limiting process, and the linear part at lower frequency corresponds to the diffusion process. The impedance values were fitted to standard Randle's equivalent circuit (inset of Fig. 2B) comprising the ohmic resistance of the electrolyte solution (R_s), charge transfer resistance (R_{ct}), surface double-layer capacitance (C_{dl}), and Warburg impedance (Z_w). In accordance with the voltammetric results above, the bare GCE generated an interfacial R_{ct} of 1558 kΩ with a straight line at low frequency and a semicircle portion at high frequency. After the modification of rGO on the GCE surface, the semicircle part on the impedance spectrum increased greatly with R_{ct} of 6,911 kΩ, reflected in the impedance results as the increase in the diameter of the semicircle at high frequency. The obvious change in the diameter of the semicircle portion indicates that rGO decelerates the electron transfer of $[Fe(CN)_6]^{-3/-4}$ because GO derivatives act as a hindering layer for electron transfer as described above. The further modification of GLOB on the GCE/rGO leads to a considerable increase of R_{ct} to 7774 kΩ, which indicates that the GLOB layer shows a blocking effect for the electron transfer of the redox probe, in agreement with CV results.

Electrochemical response of GCE/rGO/GLOB electrode surface for D- *and* L-*MA*

In voltammetric measurements, the bare GCE showed no electrochemical response for the MA enantiomers in the polarization window. After the GCE was modified with rGO and GLOB, the obtained electrodes (GCE/rGO and GCE/rGO/GLOB, respectively) gave rise to an extension at the anodic range of the polarization window for each step, as can be seen in Fig. 3A. Whereas GCE/rGO also did not exhibit any oxidation peak for each enantiomer, GCE/rGO/GLOB electrode presented excellent electrochemical results. Here the electrochemical discrimination of MA enantiomers was achieved by using GLOB that has been described as a chiral selector for MA [29]. Unlike the related study, as can be easily seen in Fig. 3B, D- and L-MA enantiomers exhibit well-defined strong

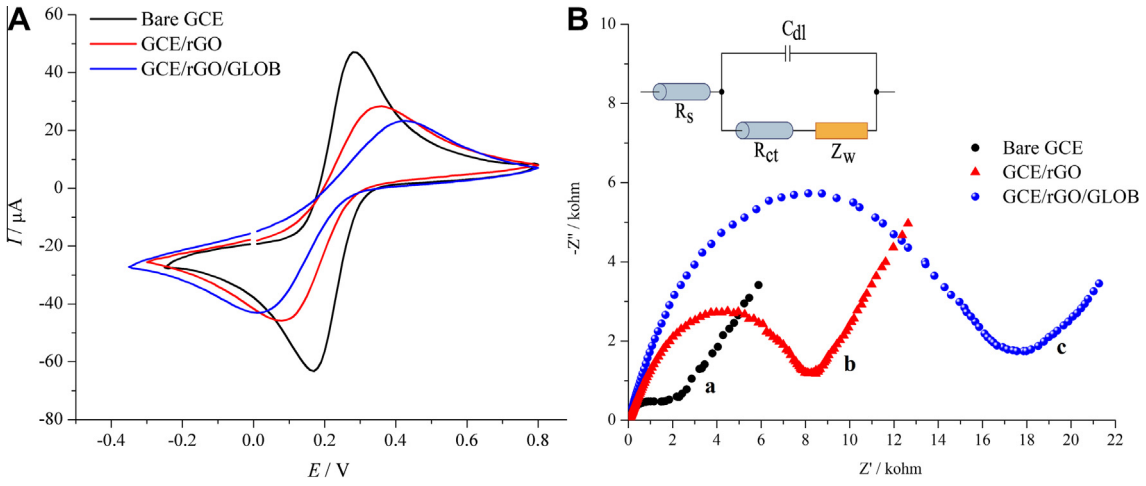


Fig.2. Cyclic voltammograms (A) and Nyquist diagrams obtained at bare GCE (a), GCE/rGO (b), and GCE/rGO/GLOB electrode (c) (B) in the presence of 1 mM $[Fe(CN)_6]^{-3/-4}$ in A–PBS solution (pH 7.01). The inset is Randle's equivalent circuit used to model impedance data in the presence of the redox couples.

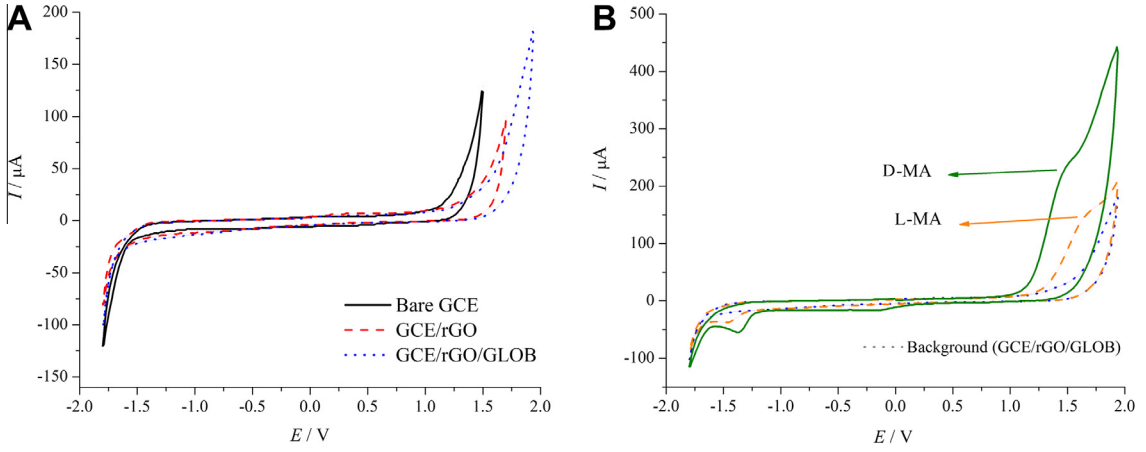


Fig.3. (A) Background voltammograms obtained at bare GCE, GCE/rGO, and GCE/rGO/GLOB electrode. (B) Voltammograms obtained at GCE/rGO/GLOB electrode in the absence and presence of D- and L-MA in A–PBS solution.

irreversible oxidation peaks at different potential values of 1.47 and 1.71 V, respectively, and exhibit weak reduction peaks at potential values of −1.37 and −1.48 V, respectively, at the GCE/rGO/GLOB surface. The obtained discrimination of MA enantiomers at different anodic potentials can be attributed to the different binding affinity of GLOB through the hydrogen bonds, hydrophobic interactions, or other actions as reported previously [29,30]. Because the chiral molecular recognition depends on the difference in stability constants of diastereomeric complexes with applied selectors and a different interaction rate of enantiomers with selector [31], electrochemical discrimination of enantiomeric pairs can be achieved by the selection of a chiral selector incorporated into the electrode surface. In accordance with this aspect, the discrimination of the enantiomeric pairs of MA can be attributed to the rGO/GLOB layer in which GLOB provides direct enantioselective discrimination of D- and L-MA. rGO not only provides a large surface for stabilization of GLOB at the electrode surface but also separates the analytical signal occurring at the end of the interaction between GLOB and D- or L-MA at different oxidation potentials. Due to achieving separation of oxidation peak potentials of D- or L-MA enantiomeric pairs, GCE/rGO/GLOB electrode possessing a chiral discrimination between D- and L-MA can be used as a direct electrochemical biosensor for discrimination of MA enantiomers.

Fig. 4 shows the additional CV measurements performed to gain an insight into the relationship between the peak current and the concentration in the range of 2 to 10 mM of D- and L-MA. As presented in the inset of Fig. 4, the oxidation peak current values of

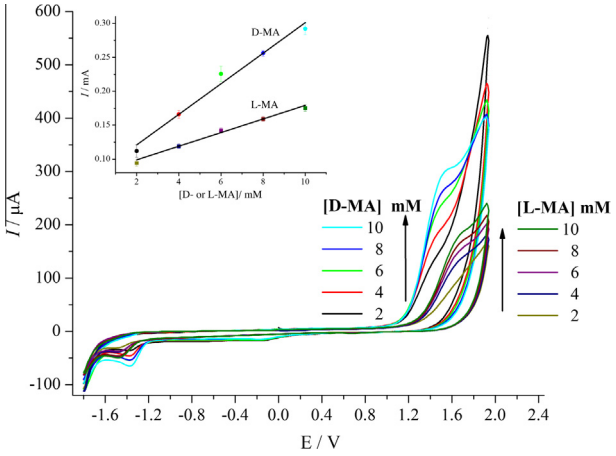


Fig.4. Cyclic voltammograms of increasing amounts of D-MA and L-MA (2–10 mM) at GCE/rGO/GLOB electrode. Scan rate = 0.1 V s^{-1}. The inset shows the linear relationship between the enantiomer concentrations of MA and the oxidation peak currents.

D- and L-MA are directly proportional to the concentration for the indicated range. Although the oxidation current values of D- and L-enantiomers increase with the increasing amounts of concentration, D-enantiomer gives higher current responses than the corresponding concentration of L-enantiomer at the GCE/rGO/GLOB electrode surface. This can be attributed to the difference of molecular interaction of MA enantiomers with GLOB causing a difference in match degree between GLOB and D- and L-MA.

Optimization of experimental conditions

Effect of pH

Due to the importance of the pH value for the activity of immobilized proteins, the electrochemical response of the electrode based on GLOB was investigated in the pH range from 5.2 to 8.2. As shown in Fig. 5A, pHs 6.2 and 7.2 have almost the same current values and pH 8.2 has the lowest current responses. At the higher pH values, the well-defined voltammetric response was not obtained. For pH 4.2, the current response is higher than those for the other pH values because the protonated form of MA enantiomers can be easily oxidized. However, the protein may be denatured at low pH values. For this reason, pH 7.2 was selected as the optimum experimental acidity for all enantioselective discrimination experiments.

Effect of amount of rGO

The relationships between the oxidation peak current (I_p) of MA and the amount of rGO on the GCE/rGO/GLOB electrode were investigated by CV. As presented in Fig. 5B, the I_p clearly decreased with the amount of rGO from 1 to 5 drops of 5 μl rGO for D-MA. However, the I_p increased with the modification of 2 drops of 5 μl rGO, whereas it decreased after modification of the surface with 3 drops of rGO for L-MA. The increase may be ascribed to homogeneous modification of rGO on the electrode surface, and the decrease may be attributed to the thicker film of rGO hampering the electrical conductivity. The current decrease with increasing amounts of rGO may be attributed to the mass deposition of rGO in basal plane characteristics on the electrode surface, as depicted by Brownson and coworkers [32]. For this reason, the volume of rGO suspension on the surface was chosen as 3 drops of 5 μl in this work due to the most suitable homogeneous surface.

Effect of incubation time in GLOB solution

Here the effect of incubation time on the oxidation peak current response (I_p) of MA on the GCE/rGO/GLOB electrode was investigated in the range of 3 to 72 h. Fig. 5C shows the I_p values, which remained stable up to 48 h for L-MA, whereas I_p exhibited a maximum at 24 h for D-MA. I_p decreased after incubation for 48 h. This result may be attributed to the thickness of GLOB wrapped homogeneously by the sheets of rGO on the surface until 24 h, and after that time so much GLOB accumulates on the surface that it alters the electron transfer to the electrode surface. For these reasons, the incubation time was kept at 24 h for the GCE/rGO/GLOB electrode in all electrochemical experiments.

Electrochemical discrimination of MA enantiomers at GCE/rGO/GLOB electrode

Due to MA generally coexisting in enantiomeric pairs in biological fluids and the fact that they show similar electrochemical behaviors at untreated carbon-based electrodes, it is important to investigate the effect of one enantiomer to the other. For this

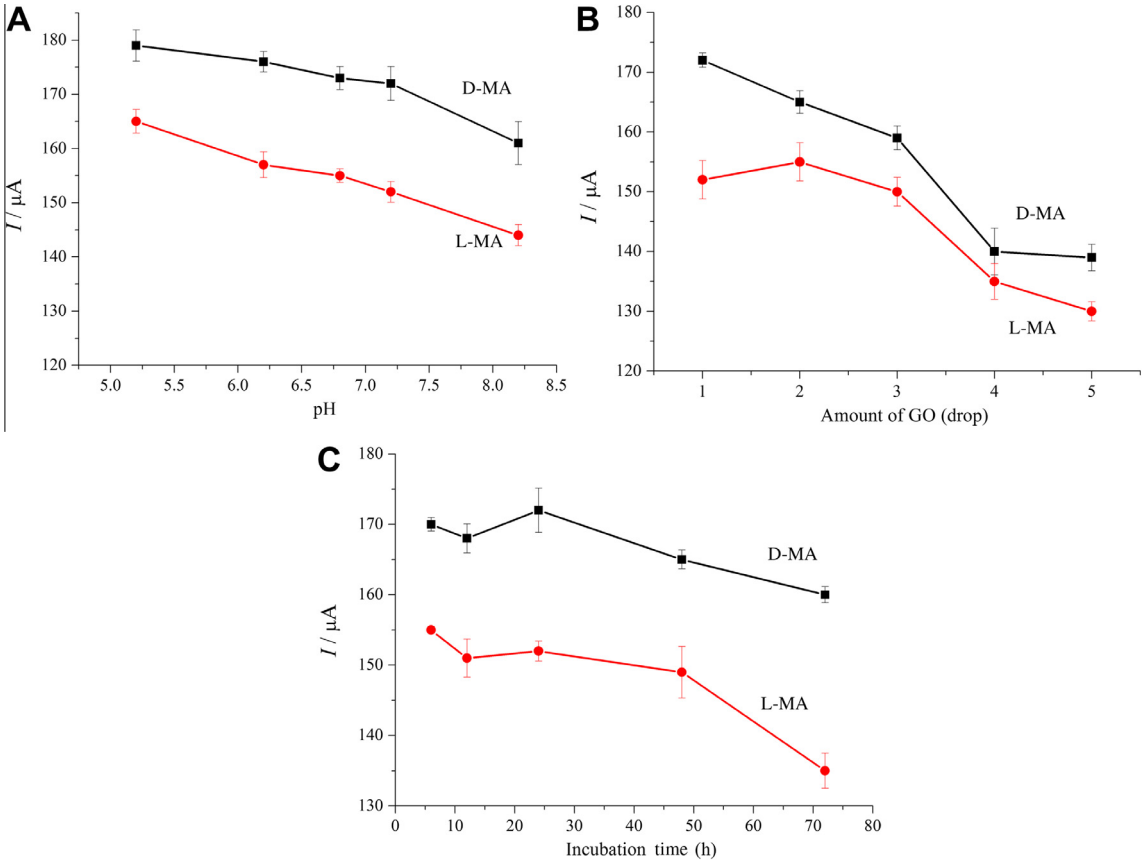


Fig.5. Changes of the oxidation peaks with pH (A), amount of the added GO drop (B), and incubation time (C). Scan rate = 0.1 V s^{-1}.

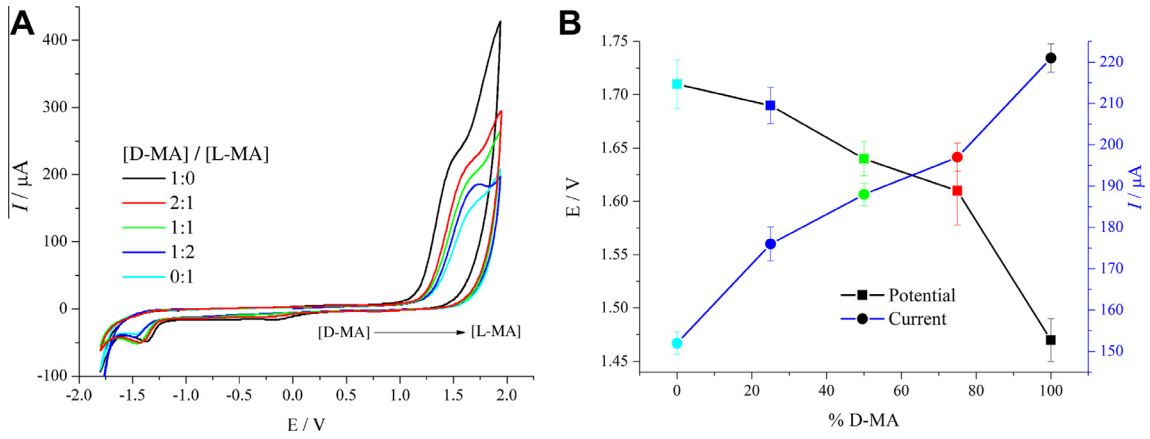


Fig.6. (A) Voltammograms of enantiomeric mixtures of a concentration ratio of D- and L-MA at GCE/rGO/GLOB electrode. (B) Changes of both the oxidation potential and the peak current with the percentage of D-MA.

reason, the GCE/rGO/GLOB electrode was used for the different concentration ratios (1:0, 2:1, 1:1, 1:2, and 0:1, c/c), as can be seen in Fig. 6A. The oxidation peak current value of D-MA decreased, whereas the oxidation peak potential shifted to the oxidation potential value of L-MA as increasing amounts of L-MA, as shown in Fig. 6A. Moreover, the oxidation of 1:1 (c/c) enantiomeric mixture occurs at 1.64 V, which is approximately the mean of the oxidation peak potential values of D- and L-MA enantiomers, indicating that there is a racemic mixture in the medium. In this manner, a linear calibration curve for the percentage of L-MA is shown in the inset of Fig. 6B in order to explain the effect of enantiomeric forms to each other. It is important to note that this result indicates that the GCE/rGO/GLOB electrode gives an opportunity for not only enantiomeric discrimination of MA pairs but also the determination of percentage of one enantiomeric form in a medium.

The stability, reproducibility and repeatability of the GCE/rGO/ GLOB electrode

The stability of the GCE/rGO/GLOB electrode was investigated by CV in a constant concentration of D- and L-MA (6.0 mM) over a period of 15 days. It was observed that during the first 5 days the current responses almost remained stable and showed a decrease in current density of approximately 7.1 and 9.2% of initial values, respectively, at the end of 15 days. The decrease in the current response may be attributed to the removal of GLOB from the electrode surface.

The reproducibility of the GCE/rGO/GLOB electrode was investigated by successive detection of 6.0 mM D- and L-MA with three independently prepared electrodes, and relative standard deviations (RSDs) were calculated as 3.8 and 4.1%, respectively. The repeatability of modified electrodes was evaluated by examining the voltammetric responses three times by using the same electrode, and RSDs were calculated as 5.8 and 4.9%, respectively.

Conclusions

Here we have presented the modified electrode (GCE/rGO/GLOB) for discrimination of MA enantiomers. The GCE/rGO/GLOB was characterized by SEM, EIS, and CV. The electrochemical response of the modified electrode for discrimination of MA enantiomers was investigated by CV. The results showed that GLOB exhibits enantioselective interaction with MA enantiomers and that the reduced GO layer on the surface not only provides a large surface for stabilization of GLOB but also separates the analytical signal occurring at the end of the interaction between GLOB and D- or L-MA. When measurements were performed to analyze D- and L-MA in a mixture, the percentage of an enantiomeric form was successfully determined, suggesting that this sensor can be used for chiral mixtures of MA. The GCE/rGO/GLOB is very simple, cheap, and not time-consuming to produce, and it provides an opportunity to make analyses for discrimination of D- and L-MA. To the best of our knowledge, it is the first time that direct chiral discrimination of MA enantiomers has been achieved at different redox potentials by the electrochemical technique in the same mixture. This study is expected to be a useful and promising platform for direct electrochemical discrimination of different chiral target materials by employing other chiral selectors.

Acknowledgments

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