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Development of a Graphene Based Electrochemical Sensor for the Determination of 9-hydroxyrisperidone in Human Blood Plasma

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This work reports the preparation of a 9-hydroxyrisperidone amperometric biosensor using a glassy carbon electrode (GCE) modified with reduced graphene oxide wrapped hierarchical TiO_2 (RGO-TiO_2) nanohybrid. In addition, the electro catalytic behaviour of the biosensor to the oxidisation of 9-hydroxyrisperidone was studied using amperometric and cyclic voltammetry (CV) measurements. The low oxidation potential and enhanced current response of the RGO-TiO_2 modified GCE resulted from the synergistic effects of TiO_2 and RGO. For the analysis of the 9-hydroxyrisperidone, the developed biosensor has a low limit of detection (LOD) of 0.52 ng/mL and a wide linear detection range of 1 to 1500 ng/mL. Therefore, our developed biosensor had the potential for use in the practical detection of 9-hydroxyrisperidone in human blood plasma specimens.

Keywords: 9-hydroxyrisperidone; TiO_2; Graphene; Human blood plasma; Electrochemical sensor

1. INTRODUCTION

As an atypical antipsychotic, risperidone acts via selective antagonism of serotonin 5-HT2 and dopamine D2 receptors [1-3]. Risperidone has a lower potential to cause extrapyramidal side effects compared to classical antipsychotics; it is effective in treating positive and negative symptoms of schizophrenia [4, 5]. Furthermore, it also has a role in the treatment of bipolar and schizoaffective disorders, as shown in recently reported open trial studies [6, 7]. Extensive 9-hydroxylation is often found for risperidone in the liver via cytochrome P450 isoenzymes (CYP), whereas 7-hydroxylation and *N*-dealkylation become less significant [8, 9]. CYP2D6 is the main catalyst for the generation of 9-hydroxyrisperidone [10-12], while the engagement of CYP3A4 has been reported in recent vitro studies [13]. Considering the similar pharmacological activity of 9-hydroxyrisperidone and the parent compound, the “active moiety” usually refers to the sum of their plasma concentrations [14]. The

influencing factors on the plasma levels of the active moiety are impaired renal and hepatic function, CYP2D6 genotype, age, and a concomitant administration of other drugs [15, 16]. Although the plasma concentrations have not been reported to obviously correlate with the clinical effects [17, 18], it is still essential to monitor the plasma 9-hydroxyrisperidone and risperidone levels in certain cases.

Few studies have been reported for the analysis of 9-hydroxyrisperidone and risperidone in human serum or plasma and those include: specific radioimmunoassay (RIA) [19], high-performance liquid chromatography (HPLC) analysis with either ultraviolet [20, 21] or electrochemical [20, 22] determination after liquid–liquid extraction [23-25] and solid-phase extraction [26, 27]. A few of the HPLC routes are characterised by low LOD or low limit of quantitation (LOQ), although they require a long operation time. Specifically, the specimen cleaning involves a multi-step liquid–liquid extraction process that requires long procedures and/or reduced recovery [28, 29]. Additionally, some measurements suffer from prolonged chromatographic runs [11, 30].

With the development of nanotechnology and nanoscience, increasing attention has been paid to the preparation of point-of-care diagnostic tools for food safety, global health, and environmental monitoring [31, 32]. For the analysis of aptamers, PNA, smRNA, pathogens, etc., many excellent multifunctional nanomaterials have been reported [33, 34]. Functional groups such as carboxyl, carbonyl, epoxide, and hydroxyl are present on the planes/edges of a GO sheet and have been used to accelerate the conjugation of biomolecules or proteins during the preparation of biosensors [35-37].

Reduced graphene oxide (RGO) can be obtained by tuning the electrical conductivity of graphene oxide (GO) through chemical reduction. The RGO has a high density of edge-plane-like defects in addition to a large surface area, which allows for a more rapid heterogeneous charge transfer. Therefore, the electron carrier mobility and electrochemical features of RGO are higher than that for GO [38-40]. Additionally, RGO is also a mechanically flexible, synthetically inexpensive and easily processed material. As such, it has been utilised extensively in many different fields, such as photodetectors, photovoltaics, field effect transistors, biosensors, etc. Anatase TiO_2 (ant-TiO_2), is non-toxic, has high biocompatibility, is inexpensive, has distinct oxidisation properties, has long-term stability and has gained wide application [19]. In addition, TiO_2 nanoparticles are also capable of adsorbing desired proteins, as well as efficiently coordinating charge transfer between an electrode and protein molecules. The preparation of ant-TiO_2 and an RGO nanohybrid has been reported for the production of a potential electrochemical immunosensor [41, 42]. These two materials can be integrated via a direct self-assembly route, where the van der Waals interactions between the oxygen moiety (on the ant-TiO_2 surface) and the graphene basal plane would be enhanced. The electrochemical performance would also be promoted after homogeneously distributing the ant-TiO_2 nanoparticles onto the multi-layered RGO.

In the present report, a TiO_2 incorporated RGO nanohybrid (RGO-TiO_2) was successfully prepared using a simple wet chemical technique. The electro catalytic activity of our developed RGO-TiO_2 modified glass carbon electrode (GCE) to the analysis of 9-hydroxyrisperidone was remarkable, with a low limit of detection (LOD) and a wide linear range. Additionally, the proposed biosensor was applied to the detection of human blood plasma specimens.

2. EXPERIMENTS

2.1. Chemicals

Graphene oxide powder was purchased from JCNANO, INC. Acetylcholine (Ach), glucose, 3-hydroxytyramine hydrochloride (DA), uric acid (UA), poly(diallyl dimethyl ammonium chloride) (PDDA, 20 wt.%), titanium butoxide, ascorbic acid, and 9-hydroxyrisperidone were purchased from Sigma-Aldrich. All reagents were analytical grade and used without further purification. For the preparation of the phosphate buffer solution (PBS), a 0.1 M solution of K_2HPO_4 and KH_2PO_4 was mixed together to a target pH value. Deionised water, 18.2 MΩ cm milli-Q, was used for all the following measurements.

2.2. Preparation of RGO-TiO_2 nanocomposite

A simple hydrothermal strategy (Lui et al. 2013) was modified and used for the preparation of TiO_2 nanoballs in our case. In brief, acetic acid (20 mL) was mixed with titanium butoxide (2 mL) and stirred for 30 min. The obtained mixture was transferred to a 50 mL Teflon-lined stainless steel autoclave, followed by heating up to 150 °C in an oven for 10 h. After cooling to ambient temperature, the sediment was centrifuged and collected. After washing and heating at 500 °C, the TiO_2 nanoballs were produced. For the synthesis of the RGO-TiO_2 nanohybrid, 100 mg of the prepared TiO_2 nanoballs were dispersed into 20 mL water and sonicated; this was followed by the addition of PDDA (2 mL), and that mixture was stirred for 2 h. After centrifugation and a water wash, the PDDA functionalised TiO_2 was obtained and followed by re-dispersion into 20 mL of water. This PDDA functionalised TiO_2 was then mixed with 2 mL of a 1 mg/mL GO dispersion and stirred for 60 min. This dispersion was further mixed with an ammonia solution (2 mL), transferred to a 50 mL Teflon-lined stainless steel autoclave and heated at 120 °C for 2 h. After centrifugation, the RGO-TiO_2 nanohybrid was collected.

2.3. Characterization

The crystal structure of the prepared samples was characterised using an X-ray diffractometer (D8 –Advance XRD, Bruker, Germany) with Cu Kα radiation. Electrochemical measurements were carried out based on GCE using a CH Instruments 660A electrochemical Workstation (CHI-660 A, CH Instruments, Texas, USA). After polishing using an alumina-water slurry, the GCE was rinsed with ethanol and water. The surface of the GCE was modified by dropping 7 μL of the 1 mg/mL catalyst dispersion onto the GCE, followed by drying at ambient temperature. The workstation was equipped with a three-compartment geometry, where the reference and auxiliary electrodes were Ag/AgCl (3 M KCl) electrodes and a platinum wire. All electrochemical experiments were performed at ambient temperature.

2.4. Blood sample preparation

Blood specimens were collected in heparinised tubes, followed by immediate centrifuging at 3000 *g* for 10 min. Prior to testing, the plasma was stored at −20 °C. A 1 mL plasma sample was mixed with NaOH (1 mL, 2 *M*) and clozapine (10 µl, 20 µg/ml; internal standard (I.S.)). After a 10 s vortex-mix, the tubes were added to 4 mL of 99:1 diisopropyl ether–isoamylalcohol (v/v; extraction solvent). The mixed solution was shaken for 10 min and centrifuged for 10 min at 4 °C and 3000 *g*. The tubes containing 150 µL of 0.1 *M* KH_2PO_4 (pH 2.2; with 25% H_3PO_4) were mixed with the organic phase at 250 cycles/min for 5 min, followed by centrifuging for 2 min at 3000 *g*. The upper organic layer was carefully aspirated for analysis.

3. RESULTS AND DISCUSSION

The RGO-TiO_2 was characterised via XRD and is shown in Fig. 1. The characteristic peaks observed at 2 θ of 25.1°, 37.3°, 47.7°, 54.2°, 54.7°, 62.4°, 68.1°, 70.7° and 75.1° resulted from the (101), (004), (200), (105), (211), (204), (116), (200), and (215) crystallographic planes of anatase, respectively, according to Fu and co-workers (2014). It was observed that all samples were primarily composed of the anatase TiO_2 phase and showed no diffraction peaks of a GO layered structure; this might be due to the disappearance of layer-stacking regularity after reduction or the low amount and relatively low diffraction intensity of graphene [43]. In addition, a broad peak overlapping the (101) plane of anatase was observed and suggested the incorporation of TiO_2 with the exfoliated RGO sheets.

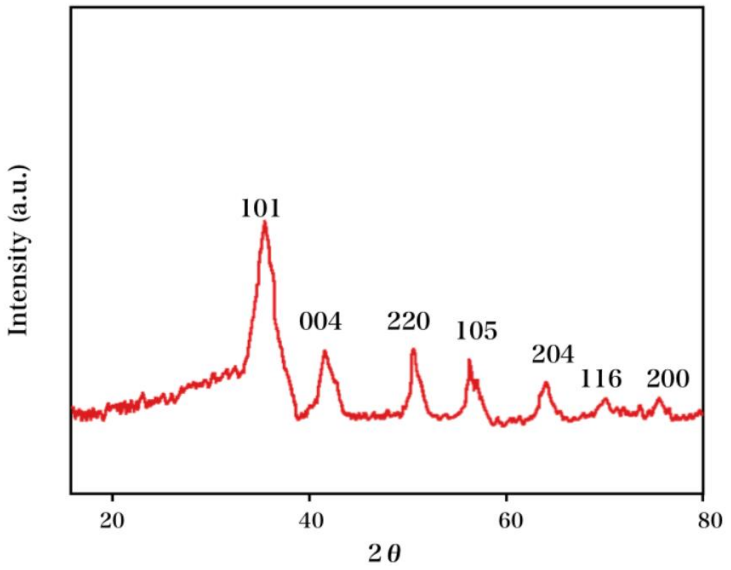

Figure 1. XRDs recorded for RGO-TiO_2 nanohybrid.

A comparative study was carried out on the electrocatalytic detection performance of the bare, TiO_2, and RGO-TiO_2 modified GCE in 0.1 M of pH 8 PBS containing 50 ng/mL 9-hydroxyrisperidone. The cyclic voltammograms (CVs) in Fig. 2 displayed no significant current response for 9-hydroxyrisperidone over an applied potential range of –0.2 to 0.2 V, whereas the TiO_2 modified GCE showed a direct oxidation peak of 9-hydroxyrisperidone at an applied potential of 0.08 V. This indicated an enhanced electrochemical reaction rate on the surface of the electrode in the presence of the as-prepared TiO_2. Additionally, a comparable CV shape was found at the GCE after modification with RGO-TiO_2, with an enhanced current and decreased oxidation peak potential (0.02 V). This suggested that the catalytic oxidation of 9-hydroxyrisperidone could be enhanced after the TiO_2 was incorporated into the RGO.

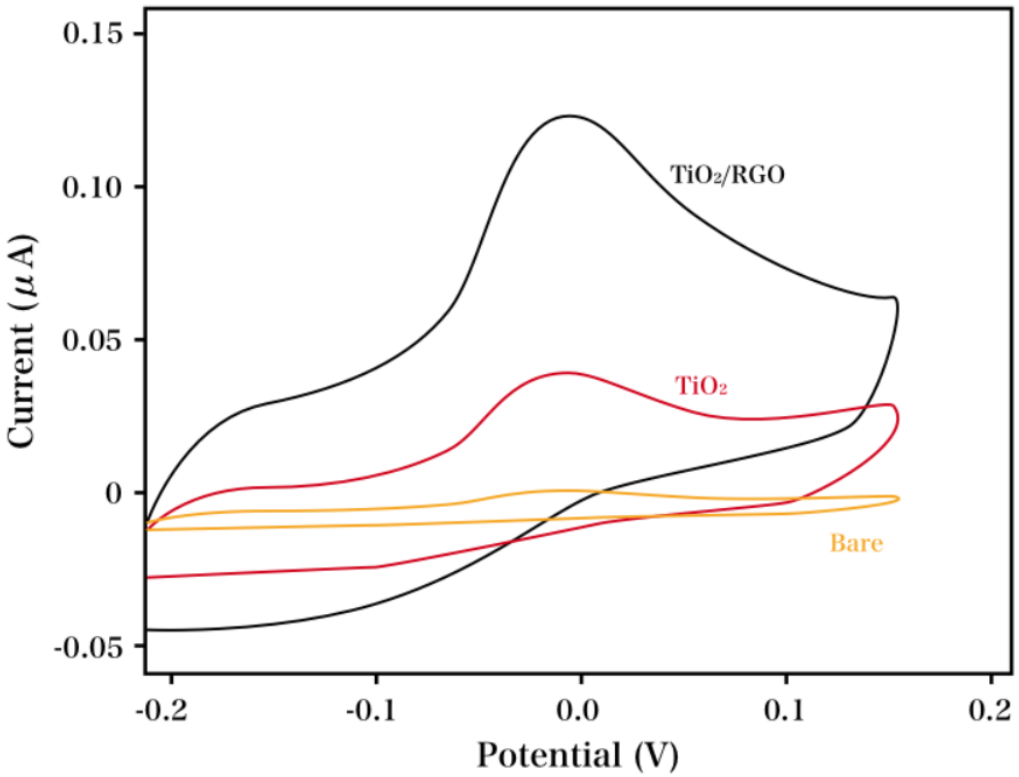

Figure 2. CVs recorded for the bare GCE, TiO_2 modified GCE, and RGO-TiO_2 modified GCE in 0.1 M PBS containing 50 ng/mL of 9-hydroxyrisperidone (scan rate of 50 mV/s).

Fig. 3 shows different peak currents of 9-hydroxyrisperidone using the RGO-TiO_2 modified GCE with varying scan rates. The oxidation current increased as the scan rate increased. The scan rate is linearly related to the oxidation current, obeys the following equation: I_{pa} (μA) = 0.84705v + 75.77 (R^2 = 0.997), suggesting that the electrode surface reaction is adsorption-controlled. Additionally, as the scan rate increased, a gradual shift to the positive direction was found for the anodic peak potential. The linearity of the logarithm of scan rate and the oxidation potential is described by the following equation: E_{pa} (V) = 0.02687 log v (V/s) + 0.02014 (R^2 = 0.9904). Two electrons are involved in this reaction (according to the Laviron's equation), which is consistent with a previous study [44].

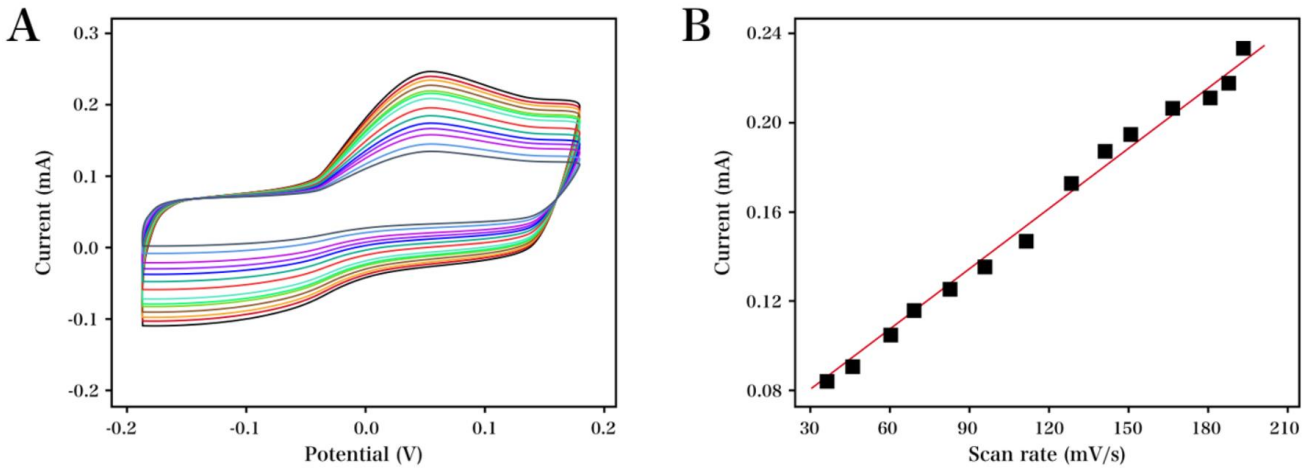

Figure 3. (A) CVs recorded for the RGO-TiO_2 modified GCE in 0.1 M PBS containing 50 ng/mL of 9-hydroxyrisperidone. Scan rate: 20—200 mV/s; (B) Plots of peak currents vs. the scan rate.

The influence of the electrolyte pH on the oxidisation of the 9-hydroxyrisperidone at the modified electrode has been reported previously. Only a non-significant dependence on pH value was observed through CV measurement, which was consistent with the result of a previous report using carbon electrodes at an applied potential [45]. The influence of pH (4–10) on the analysis of the 9-hydroxyrisperidone (50 ng/mL) using the RGO-TiO_2 modified GCE is shown in Fig. 4A. As the pH increased from 5.5 to 8, a gradual increase in the current response was observed but decreased after that. Therefore, the oxidation of 9-hydroxyrisperidone was optimised at a pH of 8. These results confirmed the involvement of 2 electrons in the first step. Two oxidation steps constitute the complete oxidation processes, where the second one refers to a $2e^-$, $2H^+$ oxidation, and involves $4e^-$ and $4H^+$ overall [46-48]. In addition, we also studied the influence of modifier amount on the anodic peak current of the 9-hydroxyrisperidone.

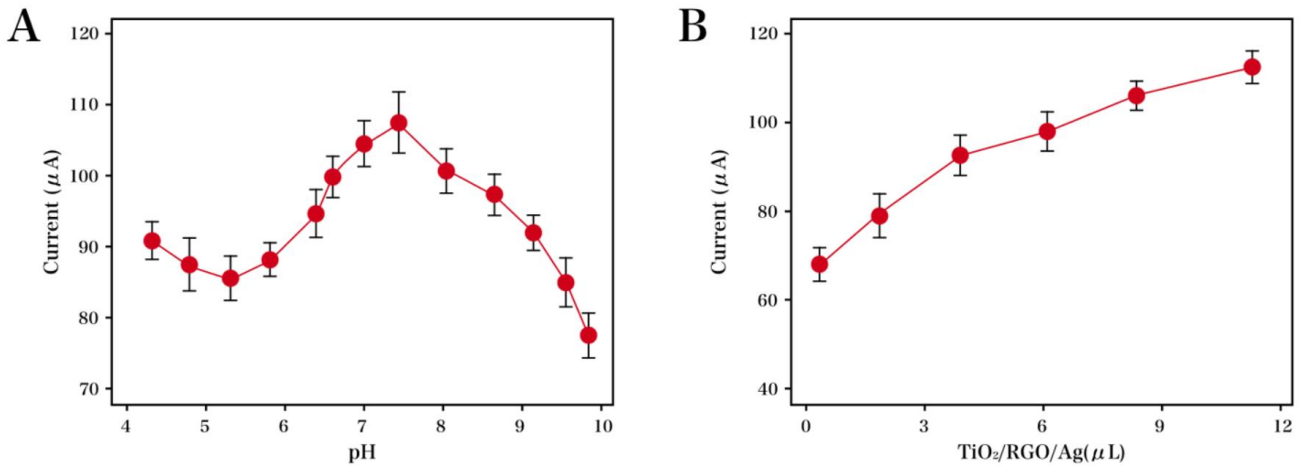

Figure 4. Plot of I_{pa} against (A) PBS pH value (B) amount of modifier for the analysis of 9-hydroxyrisperidone.

As the modifier amount increased, the anodic peak current sharply increased (Fig. 4B). The maximum peak current was found at 7 μL; the current response decreased with an additional modifier that is ascribed to the additional time it takes for the 9-hydroxyrisperidone electrons to travel through the thicker RGO-TiO_2 film.

The characteristic amperometric response of the RGO-TiO_2 modified GCE after successively adding the 9-hydroxyrisperidone is shown in Fig. 5. After each addition of 9-hydroxyrisperidone, a rapid increase in the current response was observed, followed by a steady-state within 3 s. This suggested a rapid response of the RGO-TiO_2/GCE towards the 9-hydroxyrisperidone. The relationship between 9-hydroxyrisperidone (1 to 150 ng/mL) and the current response is linear and shown in the calibration graph of the inset, with a regression equation of I (μA) = 0.09414 C(ng/mL) + 10.74 (R_2 = 0.993). Based on a signal to noise ratio of 3, the limit of detection (LOD) was determined to be 0.52 ng/mL. To allow for comparison to previous reports, the characteristics of different electrochemical sensors for 9-hydroxyrisperidone are summarised in Table 1.

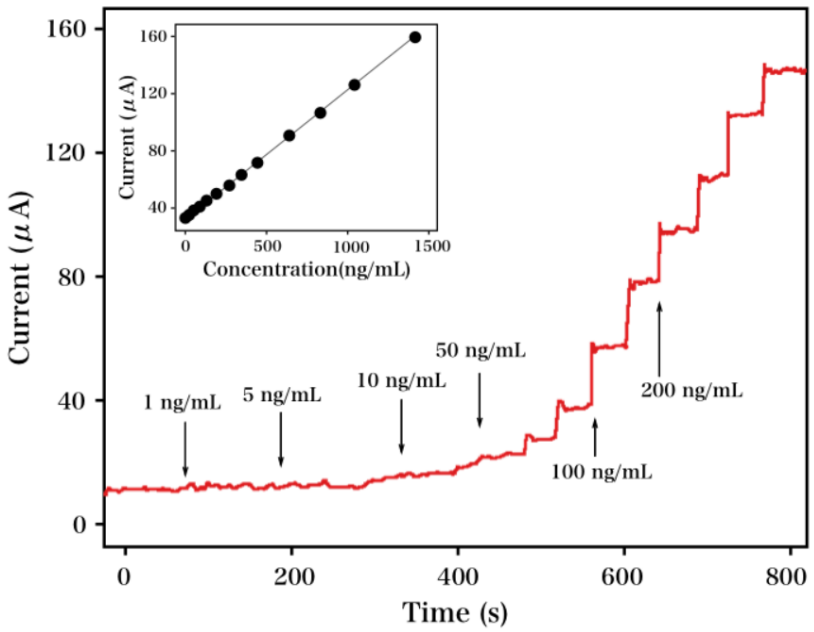

Figure 5. Amperometric response recorded for the RGO-TiO_2 modified GCE after successively adding the 9-hydroxyrisperidone at a potential of –0.02 V. Inset shows the calibration curve (concentration range: 1–1500 ng/mL).

Table 1. Comparison of the major characteristics of sensors used for the detection of 9-hydroxyrisperidone.

Electrode	Linear detection range	Detection limit	Reference
HPLC + coulometric detection	2 - 100 ng/mL	1 ng/mL	[22]
LC–MS–MS	0.1 - 250 ng/mL	—	[49]
HPLC + UV absorbance detection	5 - 100 ng/mL	2 ng/mL	[50]
RGO-TiO_2 modified GCE	1 - 150 ng/mL	0.52 ng/mL	This work

The feasibility and practicability of our developed biosensor were investigated using ten clinical human blood plasma specimens. At least three times of measurements were carried out on each specimen. Table 2 clearly shows the high reliability and feasibility of this biosensor (relative errors < 5%), and suggests a great potential for this technique to be used in clinical analysis.

Tab. 2. Detection performance of our developed technique toward clinical human blood plasma specimens.

Sample	Added (ng/mL)	Detected (ng/mL)	Recovery (%)	RSD (%)
1	20	20.41	102.05	4.7
2	40	39.78	99.45	3.1
3	80	81.02	101.27	1.9

4. CONCLUSIONS

In the present report, an RGO-TiO_2 electrode was prepared using a simple wet chemical strategy and applied to the fabrication of a biosensor towards 9-hydroxyrisperidone. The electro catalytic activity of our developed biosensor to the 9-hydroxyrisperidone oxidisation was higher than that of bare and TiO_2 modified GCEs. The lower oxidisation potential and enhanced oxidation current resulted from the synergy between TiO_2 and RGO. In conclusion, our developed biosensor exhibited a low LOD, wide linear detection range, and fast current response.

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