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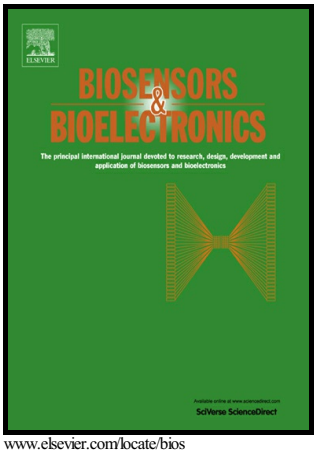

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Molecularly imprinted polymers on graphene oxide surface for EIS sensing of testosterone

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Abstract:

A novel electrochemical biosensor was developed for ultrasensitive determination of testosterone from femtomolar to micromolar levels *via* electrochemical impedance spectroscopy (EIS) measurements. The sensor features a nanosized molecularly imprinted polymer (MIP) film that was electrochemically grafted on a graphene-oxide sheets modified electrode. The detection mechanism of this senor is explained *via* the change of the interfacial impedance that derived from the recognition of the target molecule. Due to the nanosheet structure as well as the high surface area of graphene-oxide, the sensitivity of the MIP sensor is enhanced remarkably. Under an optimized condition, a wide linear range from 1fM to 1μM (1×10^{-15} mol L^{-1} to 1×10^{-6} mol L^{-1}) and a detection limit of 0.4fM (4.0×10^{-16} mol L^{-1}) was obtained. This composite film presented a good selectivity over structurally similar steroid hormones, and a long term stability in room temperature for the detection of testosterone. Considering these advantages, the MIP/GO electrochemical biosensor could be a substitute of testosterone immunosensor, and may be further extended to the detection of other endogenous substances.

Keywords: Testosterone, Electrochemical biosensor, Molecularly imprinted polymer, Graphene-oxide

1. Introduction

Testosterone is an anabolic androgenic steroid (AAS) that playing important roles in male sexual differentiation, protein synthesis and human physical performance (Mitchell and Lowe 2009). Low testosterone level is associated with high-grade prostate cancer (Lane et al. 2008) and is a risk factor of mortality and death by cardiovascular disease (Araujo et al. 2011). Testosterone has also been used by athletes as doping substances to improve their performance. The World Anti-Doping Agency (WADA) prohibited their use to ensure fair play and to protect athletes from possible adverse side effects (Houtman et al. 2009). Accordingly, monitoring the levels of testosterone in biological fluids is of practical significance in clinical, biochemical and sports endocrinology research. Immunoassay methods based on capillary electrophoresis (Chen and Zhang 2008), electrochemiluminescence (Brandhorst et al. 2011), electrochemistry (Liang et al. 2008), and surface plasmon resonance (SPR) (Mitchell and Lowe 2009) have been reported for the detection of testosterone. Nevertheless, the employment of natural antibodies make these methods difficult to reproduce in large quantities due to the limitations of storage stability, sensitivity and detection limits. These constraints may be overcome by replacing the natural antibodies with the artificial receptors. One promising method is using molecular imprinting polymers (MIPs), a synthetic polymer with specific recognition sites, as the sensitive layer of the biosensors (Ji et al. 2015; Karimian et al. 2013; Piletsky and Turner 2002).

A MIP based electrochemical sensor can be constructed by transducing the recognition signal into an electrochemical signal. Electrochemical detecting methods offer the merits of high detection sensitivity as well as simplicity, low cost, accuracy, and a wide dynamic concentration range for the detection of various analytes (Bahadır and Sezgintürk 2015; Eguilaz et al. 2010). The MIP layer plays a role of sensitive element of an electrochemical sensor. The recognition efficiency of the sensor, however, is dependent on the binding capacity, mass transfer rate, and shape of the MIP materials (Zhang et al. 2012). A variety of traditional

imprinted materials suffer from low recognition efficiency, as many binding sites were buried in polymeric matrix during the bulk polymerization (Alizadeh et al. 2012). This problem could be overcome by employing nanomaterial as the supporting material for MIP, owing to the advantages of forming binding sites predominantly at the material surface as well as large surface, good stability and easy functionalization (Yang and Zhao 2015). For instance, MIP materials based on Au–Ag alloy microrod (Li et al. 2016), porous Au-paper (Ge et al. 2013), gold coated Fe_3O_4 (Han et al. 2016), and carbon nanotube (Tong et al. 2013) have been proved to be able to enhance the sensitivity and affinity of the sensor. Graphene-oxide (GO) sheets, as a one-atom-thick two-dimensional monolayer of graphite with a high surface areas (Novoselov et al. 2004), are ideal supporting material for MIP materials. The graphene based materials have also exhibited great potential in the application of electrochemical sensors (Liu et al. 2015; Zhang et al. 2011).

In the present manuscript, we have designed and synthesized a novel nanocomposite of molecularly-imprinted polymer/graphene-oxide (MIP/GO) for the fabrication of a testosterone electrochemical sensor. The first key of our approach was using MIP as the sensitive layer in order to enhance the selectivity and overcome the limitations of storage stability. The second key of our approach was grafting the MIP martial on the surface of GO sheets in order to enlarge the surface area and to enhance the detection sensitivity. The third key of our approach was employing electrochemical impedance spectroscopy (EIS), a non-faradaic electrochemical method to detect the recognition signal of testosterone. EIS is particularly sensitive to surface interactions and is demonstrated as an effective method for investigating electrode processes, surface adsorption kinetics, and mass-transport parameters (Apodaca et al. 2011). However, the EIS detection of testosterone recognition on the MIP/GO composite film is still a challenge. To the best of our knowledge, this is the first time to construct such an EIS biosensor for the ultrasensitive and selective detection of testosterone.

2. Experimental section

2.1 Materials and Instruments

Natural graphite powder was purchased from Alfa Aesar, o-phenylenediamine (o-PD) and testosterone were obtained from Aladdin reagent China. All the other reagents were obtained from Sinopharm chemical reagent.

The as-prepared electrode materials were characterized using transmission electron microscopy (TEM, FEI TecnaiG^2 F20 s-twin D573), Raman spectroscopy (Horiba Jobin Yvon T64000 system with 514.5nm radiations), Fourier transform infrared spectroscopy (FT-IR, Bruker IFS55), X-ray diffraction (XRD, CuKα radiation, D/max2550VB, Rigaku), and Scanning electron microscopy (SEM, Hitachi S-4800).

Electrochemical measurements were conducted using a CHI760d workstation equipped with a three-electrode cell. A modified glassy carbon electrode (GCE, 3 mm in diameter) was used as the working electrode, a platinum wire was used as the counter electrode, and an Ag/AgCl electrode (KCl saturated) was used as the reference electrode. All potentials in the text are with respect to the Ag/AgCl reference electrode.

2.2 Preparation of the molecularly-imprinted polymer/graphene-oxide (MIP/GO)

The preparation of the MIP/GO composite was described in scheme 1.

(1) *Drop-casting GO on the electrode surface.* The graphite oxide was first synthesized from natural graphite powder using the modified Hummers method (Hummers and Offeman 1958). Graphene oxide (GO) were obtained by exfoliation of graphite oxide dispersion in a ultrasonic-bath for 30 min. Then, 5 μL of GO suspension was carefully dropped on the cleaned GCE surface and dried in atmosphere environment.

(2) *Electropolymerization of the MIP layer on the surface of GO modified electrode.* The GO modified electrode was immersed into acetate buffer solution (ABS, pH 4.5) that containing 2.0 mM monomer (o-phenylenediamine) and 0.1 mM template (testosterone). A typical electropolymerization was performed by cyclic voltammetry (CV) (30 cycles) between 0 V and 1.0 V at a scan rate of 50 mV/s. The ABS was bubbled with nitrogen before electropolymerization.

(3) *Template removal*. The template molecular (testosterone) was removed by washing in a stirring ethanol solution for 30 min. After removing the template molecular, the specific recognition sites were produced in the polymer membranes. Then, this MIP/GO modified electrode was used for the sensing application. The recognition of the target testosterone on the MIP/GO composite electrode induced the EIS signal for testosterone detection.

The non-imprinted polymer (NIP) grafted GO electrode were prepared under the same experimental conditions in each case however without adding the testosterone template.

Insert Scheme 1.

2.3 Optimizing the MIP/GO composite

Firstly, the monomer/template ratio was optimized in the range of 5, 10, 15, 20, 25, and 30 with the template fixed at 0.1mM. Secondly, the number of CV scan cycles in the MIP electropolymerization was optimized in the range of 10, 20, 30, 40, and 50 cycles. Thirdly, the incubation time for the recognition of testosterone was optimized in the range of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20min. The optimization experiments were conducted based on the EIS response of MIP/GO modified electrode towards testosterone in three concentrations of 100fM, 100pM, and 100nM. The synergistic effect of the three factors on the EIS response were optimized using Minitab 17.0^{TM} package software in the Box-Behnken model.

2.4 Electrochemical behaviour of the MIP/GO modified electrode

The electrochemical behaviour of MIP/GO modified electrode in the preparation process were characterised by CV and EIS. Both CV and EIS were conducted using a redox probe of 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1 M KCl solution. CV were conducted in the potential range between –0.2 V and +0.7 V at a scan rate of 50 mV/s. EIS measurements were carried out under an open-circuit voltage, with the frequency range of 0.01 to 100 000 Hz and the signal amplitude of 5 mV. The electron transfer resistance (Rct) values that equal to the semicircle diameter were obtained by fitting the impedance spectra to the

equivalent electrical circuits in the inset of Fig. 3(C) using ZSimpWin (Echem software). As a comparison, the electrochemical behaviour of NIP/GO modified electrode were also evaluated.

2.5 Assay procedure

The concentration of testosterone was detected by EIS measurements based on the recognition of the target molecule on the MIP/GO electrode. The MIP/GO modified electrode was first incubated for 10 min in PBS (pH 7.0) containing a certain concentration of testosterone. Then, the electrode was immersed in 10 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) and 0.1 M KCl solution for EIS measurements, which were carried out under an open-circuit voltage with the frequency range of 0.01 to 100 000 Hz and the signal amplitude of 5 mV. As a comparison, the EIS response of MIP modified electrode that without GO were also evaluated by measuring testosterone in three concentrations of 100fM, 100pM, and 100nM.

The human serum sample was pretreated before EIS sensing. Interfering proteins in serum were removed by precipitation with trichloroacetic acid (10%, w/v) and centrifugation for 15 min. The serum samples were diluted 1:10 with PBS (pH 7.0). MIP/GO electrode were dipped into the diluted sample for 10 min, followed by washing and subsequent detection.

3 Results and discussion

3.1 Preparation and optimization of the MIP/GO composite

The cyclic voltammogram for the preparation of MIP layer on GO modified electrode was shown in Fig.1(A). Distinct and irreversible anodic peaks corresponding to the oxidation of o-phenylenediamine was observed at a potential of ~0.7V in the first scan. The anodic peaks decreased considerably with increasing numbers of consecutive scan cycles, indicated that the polymer created an insulating MIP layer on the surface of GO nanosheets. During the electropolymerization, the MIP polymer membranes generated

gradually around the template molecular, and the template molecules were embedded in the polymer membranes. The presence of protonated $-NH_2$ groups of o-phenylenediamine in ABS (pH 4.5) may be responsible for interactions with the hydroxyl groups in the template molecular (Tiwari et al. 2012).

The monomer/template ratio was optimized as it plays an important role in the polymer structure and the rebinding affinity. As shown in Fig.1(B), the EIS response reached the maximum when the monomer/ template ratio was 20. It is describe that inadequate amount of the monomer resulted in the lack of crosslinker and difficulty in forming effective imprinted cavities. While the superabundant amount of the monomer will lead to high cross-linking degree and the templates embedded too deep to remove, which reducing the imprinting efficiency.

The number of CV scan cycles during the electropolymerization process was optimized as it determines the thickness of the MIP layer. When the CV scan reached 30 cycles, a relatively high EIS response was obtained in the detection of testosterone Fig.1(C). It is described that a thinner MIP layer led to less specific cavities, and a thicker MIP layer led to the active binding cites buried deep in the polymer structure and made the target testosterone difficult to reach the binding cites.

The incubation time was optimized by immersing the modified electrode into the test solution for different time intervals. Fig.1(D) exhibits that the ΔRct value increased quickly with the increasing of absorption time and then levelled off at 10 min, indicating a saturation adsorption time of 10 min. The rapid dynamic adsorption of testosterone could be ascribed to that GO nanosheets provides large amount of binding cites on the surface of the imprinted polymer film.

Insert Fig.1

The synergistic effect of the three factors (monomer/template ratio, number of CV scan cycles, and

adsorption time) on the EIS response were optimized using Minitab 17.0™ package software in the Box-Behnken model. The Box-Behnken tests require three factors with low, middle, and high levels (Table S1 in the supporting information.). The design and results of Box-Behnken tests are shown in Table S2 in the supporting information. Each test was repeated 3 times and the average Rct values were used.

The results of the Box-Behnken tests were analyzed using Minitab software. A regression equation was obtained (Equation 1) with R^2 of 93.17% , which indicates that the model is suitable for the prediction of simulated variables.

$$R_{CT} = 416 + 402.8\,A + 63.5\,B + 552\,C - 10.46\,A^*A - 0.830\,B^*B - 25.95\,C^*C + 0.145\,A^*B + 2.75\,A^*C - 1.75\,B^*C \quad \text{(Equation 1)}$$

where R_{CT} is the EIS response to 100pM of testosterone, A is the monomer/template ratio, B is the number of CV scan cycles, C is the adsorption time.

Surface plot of R_{CT} versus the interaction effect of A and B, A and C, B and C was shown in Fig.S1 in the supporting information. It is observed that middle levels of the factors resulted in relatively higher Rct values. In order to obtain the maximum Rct value, the three factors were further optimized using the response optimizer in the Minitab software. The optimization plot (Fig.S2 in the supporting information) indicates that the maximum Rct value (8509 Ω) was obtained in the optimal condition of monomer/template ratio: 20.86, number of CV scan cycles: 28.69, and adsorption time: 10.79 min.

3.2 Characterization of the MIP/GO composite

This nanosheet structure of GO introduced the high specific surface area of the electrode material and the large specific binding cites of the MIP sensor. This nanostructure was demonstrate as follows. TEM image of GO in Fig.2(A) illustrated highly monodispersed nanosheets with a size distribution from 150 to 600 nm in diameter. Raman spectroscopy of GO nanosheets in Fig.2(B) show primary peaks centered at

~1347 cm^{-1} (D band), ~1585 cm^{-1} (G band), and ~2685 cm^{-1} (2D band). The D band is usually attributed to the disorder and imperfection of the carbon crystallites, the G band is ascribed to the E_{2g} phonon of C sp^2 atoms, and the 2D band is a second order vibration caused by the scattering of phonons at the zone boundary. (Berciaud et al. 2009; Tuinstra and Koenig 1970; Wang et al. 2008). The broad 2D band at 2691 cm^{-1} indicated that GO was consist of about 5 layers (Ferrari 2007; Lotya et al. 2010). Moreover, the GO naosheets were evaluated by FT-IR and XRD as described in the supporting information. Briefly, FT-IR analysis in Fig.S3 (supporting information) indicated the presence of the oxygen-containing functional groups on GO nanosheets The XRD analysis in Fig.S4 (supporting information) confirmed the lamellar crystal structure of GO.

The morphology of GO modified electrode and MIP/GO modified electrode were illustrated by the SEM images. The crumbled and curved structure of multiple-layer GO nanosheets in micrometer domain on the electrode surface can be observed Fig.2(C). This SEM image indicated a good coverage of GO on the electrode surface. When the MIP layer was coated on the GO modified interfaces, an amorphous and more rough interface can be observed in Fig.2(D). This change in the morphology of the interfaces demonstrated that the employment of GO nanosheets as supporting material resulted in a MIP layer with a large electrode surface area, and could offers more active binding site for the recognition of target testosterone molecular.

Insert Fig.2

3.3 Electrochemical behaviour of the MIP/GO composite

Fig.3(A) shows the cyclic voltammograms of the redox probe of $K_3Fe(CN)_6/K_4Fe(CN)_6$ obtained on the bare electrode and MIP/GO modified electrode. Owning to the coating of MIP/GO layer on the electrode surface, the oxidation-reduction peak was changed from curve-a to curve-b accompanied by a remarkable decrease in the peak current. This fact indicated that the redox reaction of $K_3Fe(CN)_6/K_4Fe(CN)_6$ was

obstructed by the insulating MIP/GO layer. After washing of the MIP/GO modified electrode with ethanol solution, the redox reaction of $K_3Fe(CN)_6/K_4Fe(CN)_6$ was recovered and a pair of oxidation-reduction peak was observed (curve-c). The recovery of redox reaction indicated the template molecular was successfully removed from the MIP layer and resulted in the more available access for $K_3Fe(CN)_6/K_4Fe(CN)_6$ through the cavities in MIP/GO layer. After rebinding 1pM testosterone, a decrease in the peak current from curve-c to curved-d was observed, which was ascribed to the obstruction of access to $K_3Fe(CN)_6/K_4Fe(CN)_6$ through the MIP layer.

As a comparison, the cyclic voltammograms of NIP/GO was presented in Fig.3(B), the oxidation-reduction current decreased dramatically (curve-a to curve-b) after the coating of NIP/GO on the bare electrode, which is similar to that of the MIP/GO electrode. However, after ethanol washing and template removal, the change of oxidation-reduction current was very little (curve-b to curve-c) because almost no cavities with binding sites was obtained on the NIP/GO layer. After the NIP/GO modified electrode was incubated with testosterone (curve-d), no change on the cyclic voltammogram was observed, which indicated that NIP/GO failed to recognize the target testosterone.

EIS was used to further characterized the electrochemical behaviour of the MIP/GO modified electrode as shown in Fig.3 (C). The Rct values increased from 197 Ω (curve-a) to an extreme large value (curve-b) when the MIP/GO composite was coated on the electrode. Then, the Rct decreased to 3974 Ω (curve-c) after template removal, and increased back to a relatively higher value of 7353Ω (curve-d) after the electrode was incubated with 1pM of testosterone. This change of the Rct values verified that the MIP/GO modified electrode had good capability to recognize the target molecule, which were in accordance with the result of CV assays. Due to the high sensitivity to detect the rebinding of testosterone, the EIS method was applied to detect testosterone in different concentrations.

Insert Fig.3

3.4 Analytical performance

The MIP/GO modified electrode was used for the detection of testosterone. Fig.4(A) showed the radius of the semicircle (Rct value) increased with the increasing of testosterone concentration, indicating a sensitive response to the rebinding of testosterone. A calibration plot in Fig.4(B) revealed a linear increase of Rct values with the log concentration of testosterone in the range of 1 fM – 1 μM (1×10^{-15} mol L^{-1} – 1×10^{-6} mol L^{-1}) with a correlation coefficient of 0.9978. The calibration plot was evaluated by 3 times determination, the relative standard deviation (RSD) value in each concentration was less than 5.72%. The limit of detection (LOD) was calculated as 0.4 fM (4.0×10^{-16} mol L^{-1}) at a signal-to-noise ratio of 3.

The analytical performance of MIP/GO modified electrode was compared with that of the MIP modified electrode. As shown in Fig.4(C), the EIS response of MIP/GO modified electrode increased obviously with the increasing of testosterone concentrations (100fM ~ 100nM). However, the EIS response of the MIP modified electrode increased tardily with the increasing of testosterone concentrations. This fact indicated an enhanced detection sensitivity of MIP/GO modified electrode compared with that of MIP modified electrode, owing to the large surface area and more active binding cites that derived from GO nanosheets.

Insert Fig.4

3.5 Selectivity, reproducibility, and stability

To verify the selectivity of the MIP/GO modified electrode towards testosterone, experiments were conducted by testing the EIS response of some structurally similar interferences (estradiol, estriol, ethinyloestradiol) and endogenous substances (ascorbic acid, dopamine, uric acid, glucose). Fig. 4(D) revealed the Rct values of these structurally similar interferences were below 8.0% of that of testosterone, and the Rct values of these endogenous substances were below 6.2% of that of testosterone. The selectivity

was derived from the specific binding cites of testosterone that were formed during the co-electropolymerization of the monomer and template molecule.

The repeatability of MIP/GO modified electrode was evaluated by 11 times successive determination of 10 pM testosterone with the same electrode. The electrode exhibits a satisfying repeatability with a RSD value of 3.65%. The electrode-to-electrode reproducibility was examined using five different electrodes with a RSD value of 4.23%. After the MIP/GO modified electrode was stored for 30 days at room temperature, it kept 93.4% of its initial response. This long-term stability demonstrated advantage of MIP/GO sensor compared with the antibodies-based testosterone sensors. Moreover, the MIP/GO sensor revealed an advantage of regeneration ability. The polluted MIP/GO sensor can be regenerated by washing with ethanol solution and the response can reach 96.2% of the initial response.

3.6 Sample analysis

Diluted human serum from a male volunteer in our lab was used as the sample. The concentration of testosterone in the sample was determined as 127.8±0.9pM. The recoveries of the spiked testosterone were between 98.6–104.2% with RSD < 4.82% (as shown in Table 1).

Insert Table 1

The analytical performance of this MIP/GO sensor was compared with several methods in the recent reports. The MIP/GO sensor presented a relatively wider linear range and lower detection limit (Table S3 in the supporting information). Moreover, the MIP/GO sensor overcome the constraints of storage limitations of the immunosensors. Considering the advantages of high selectivity, sensitivity, reproducibility, stability and the regeneration ability, the MIP/GO sensor provided a promising method for the detection of testosterone in clinical diagnosis.

4. Conclusions

In summary, a novel electrochemical sensor based on the molecular imprinted polymer graphene-oxide composite was developed for the ultrasensitive detection of testosterone in biological fluids. The molecularly imprinted polymer was prepared through a simple and low-cost electropolymerization method on a graphene-oxide modified electrode. The acquired sensor exhibits excellent selectivity, high sensitivity, rational adsorption kinetics, and long-term stability, that derived from the large surface area of graphene-oxide and the abundant imprinted cites on the molecularly imprinted polymer. To the best of our knowledge, this is the first time to detect testosterone using electrochemical impedance spectroscopy based on molecularly imprinted polymers. The strategy can be further expected in the application of detecting endogenous substances, drugs, and environmental pollutants

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://

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Table

Table 1. Determination of testosterone in human serum sample (n=5)

	Found (pM)	Spiked (pM)	Recovery (%)	RSD(%)
Sample	127.8±0.9	100.0	102.0	3.24
		200.0	98.6	4.16
		300.0	104.2	4.82

Figure Captions

Scheme 1. The process for the preparation of the MIP/GO composite. (1) Drop-casting graphene-oxide sheets on the GCE surface. (2) Electropolymerization of the MIP layer on the surface of GO modified electrode. (3) Template removal and recognition of the target testosterone in different concentrations.

Fig.1 (A) Cyclic voltammograms for electropolymerization of 2.0 mM o-phenylenediamine with 0.1mM testosterone on GO modified electrode. Potential range: 0V ~ 1.0V. Scan rate: 0.05V/s. Number of scan cycles:30. The plots of the Rct values versus the monomer/template ratios (B), the Rct values versus the number of CV scan cycles (C), and the Rct values versus the incubation time of the testosterone recognition (D) that obtained *via* the EIS detection of testosterone in the concentration of 100fM (curve-a), 100pM

(curve-b), and 100nM (curve-c). (n=3).

Fig.2 (A) TEM image of GO nanosheets. (B) Raman spectra of GO nanosheets. (C) SEM image of GO modified electrode. (D) SEM image of MIP/GO modified electrode.

Fig.3 (A) Cyclic voltammograms of the bare electrode (curve-a), MIP/GO electrode (curve-b), MIP/GO electrode after washing with ethanol (curve-c), MIP/GO electrode after rebinding of testosterone (1pM). (curve-d). (B) Cyclic voltammograms of the bare electrode (curve-a), NIP/GO electrode (curve-b), NIP/GO electrode after washing with ethanol (curve-c), NIP/GO electrode after rebinding of testosterone (1pM). (curve-d). (C) Nyquist plots obtained at a bare electrode (curve-a), MIP/GO modified electrode (curve-b), MIP/GO modified electrode after template removal (curve-c), MIP/GO modified electrode after rebinding of 1pM testosterone (curve-d). The inset in Fig.3C is the equivalent circuit, where Rct is the electron transfer resistance.

Fig.4 (A) The EIS response of the MIP/GO electrode towards testosterone in the concentration of 1fM 10fM, 100fM, 1pM, 10pM, 100pM, 1nM, 10nM, 100nM, and 1µM. (B) The calibration plot of the Rct values versus the log concentration of testosterone (n=3). (C) Comparison of the EIS response between MIP/GO modified electrode and MIP modified electrode towards 100fM, 100pM, and 100nM testosterone (n=3). (D) EIS response of the MIP/GO modified electrode towards 100fM testosterone, estradiol, estriol, ethinyloestradiol, ascorbic acid, dopamine, uric acid, and glucose, respectively.

Figures

Scheme 1.

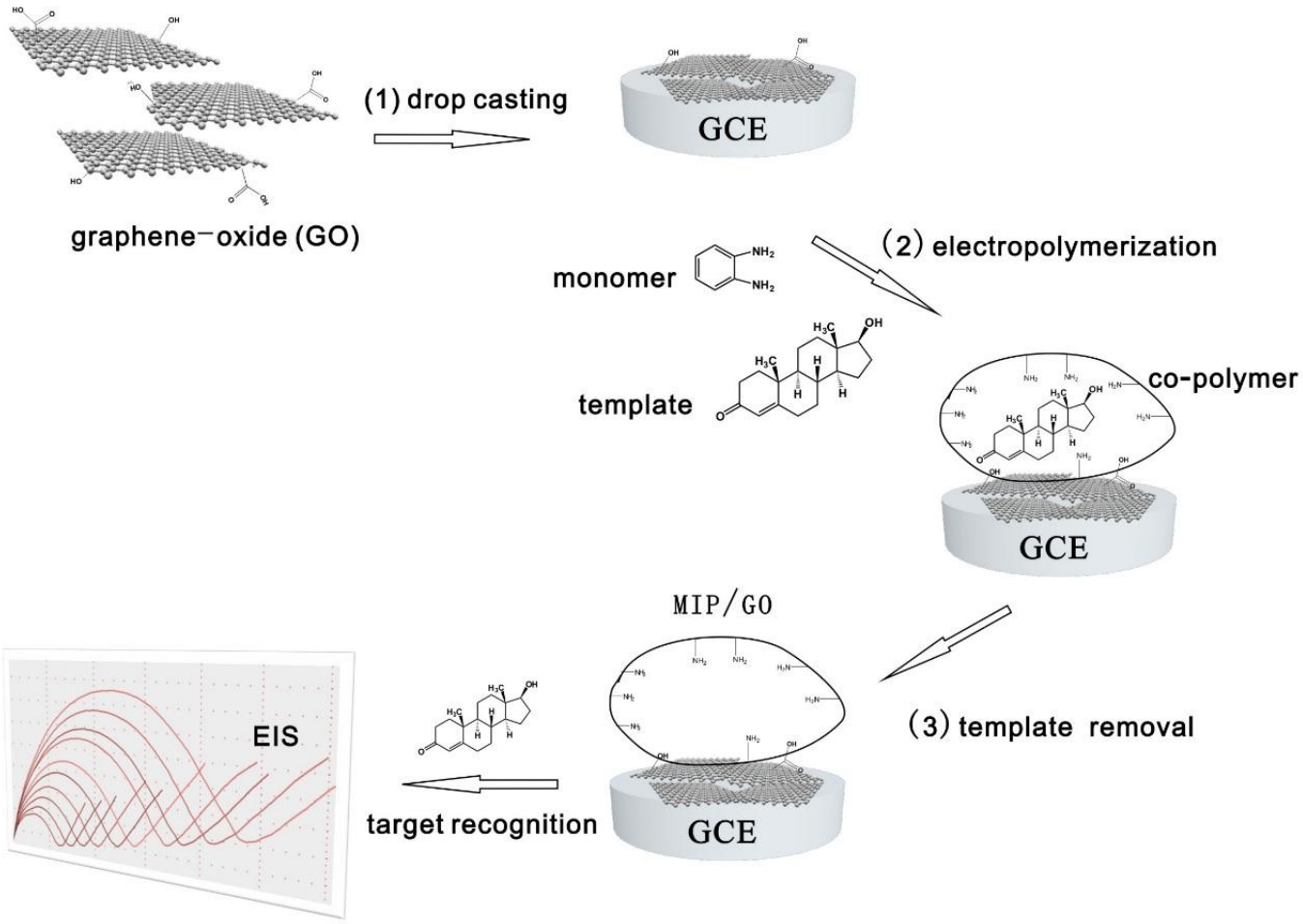


Fig.1

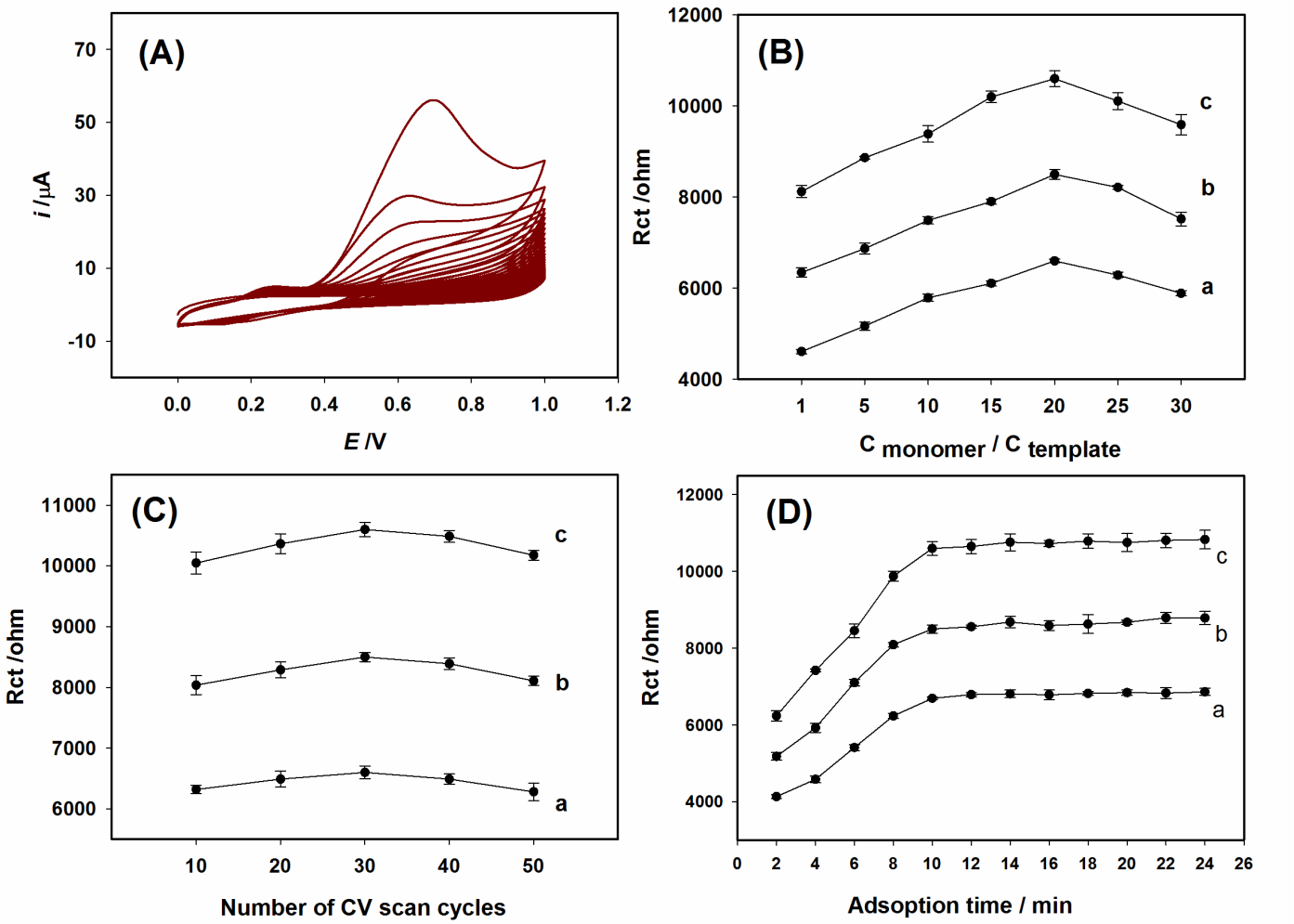


Fig.2

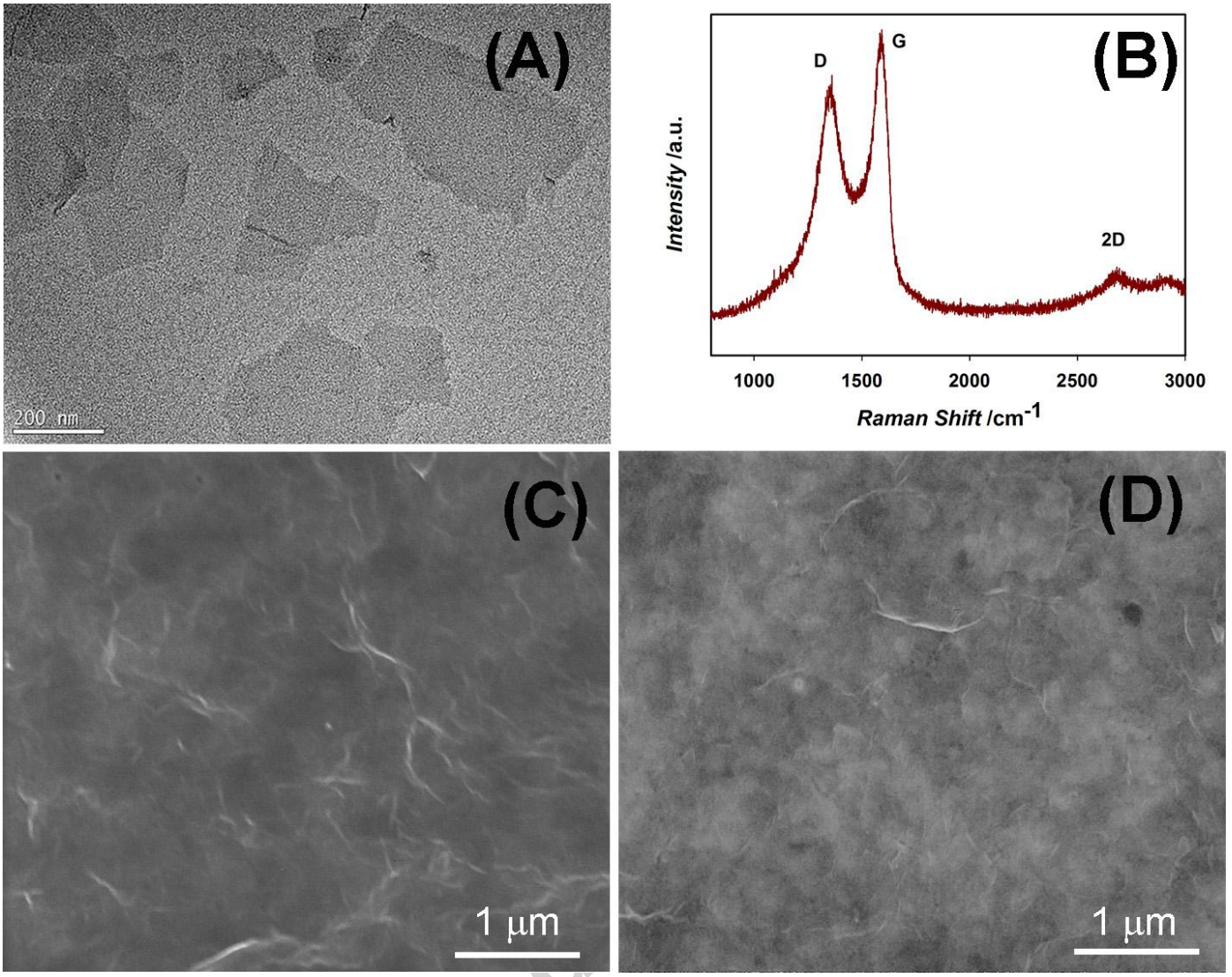


Fig.3

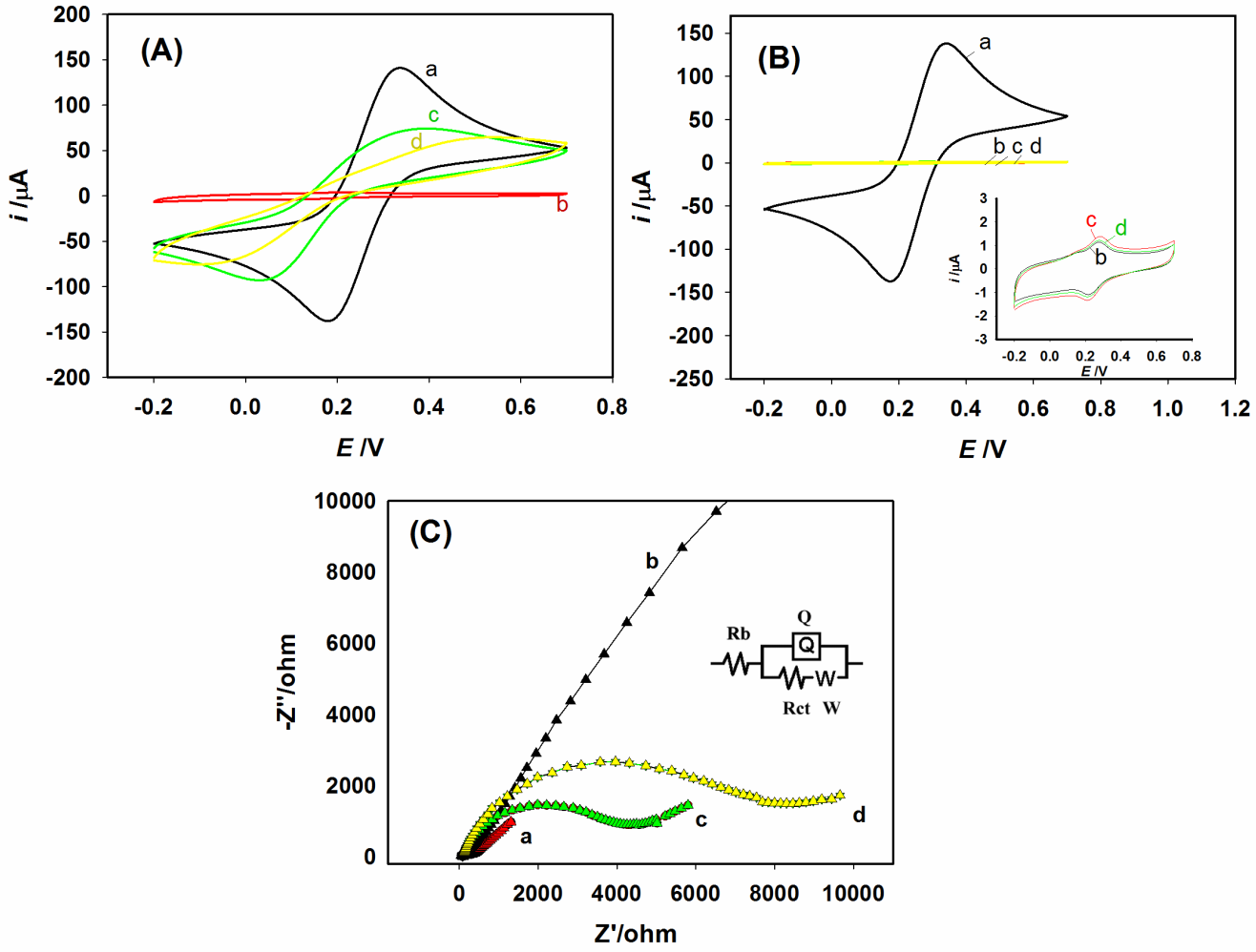
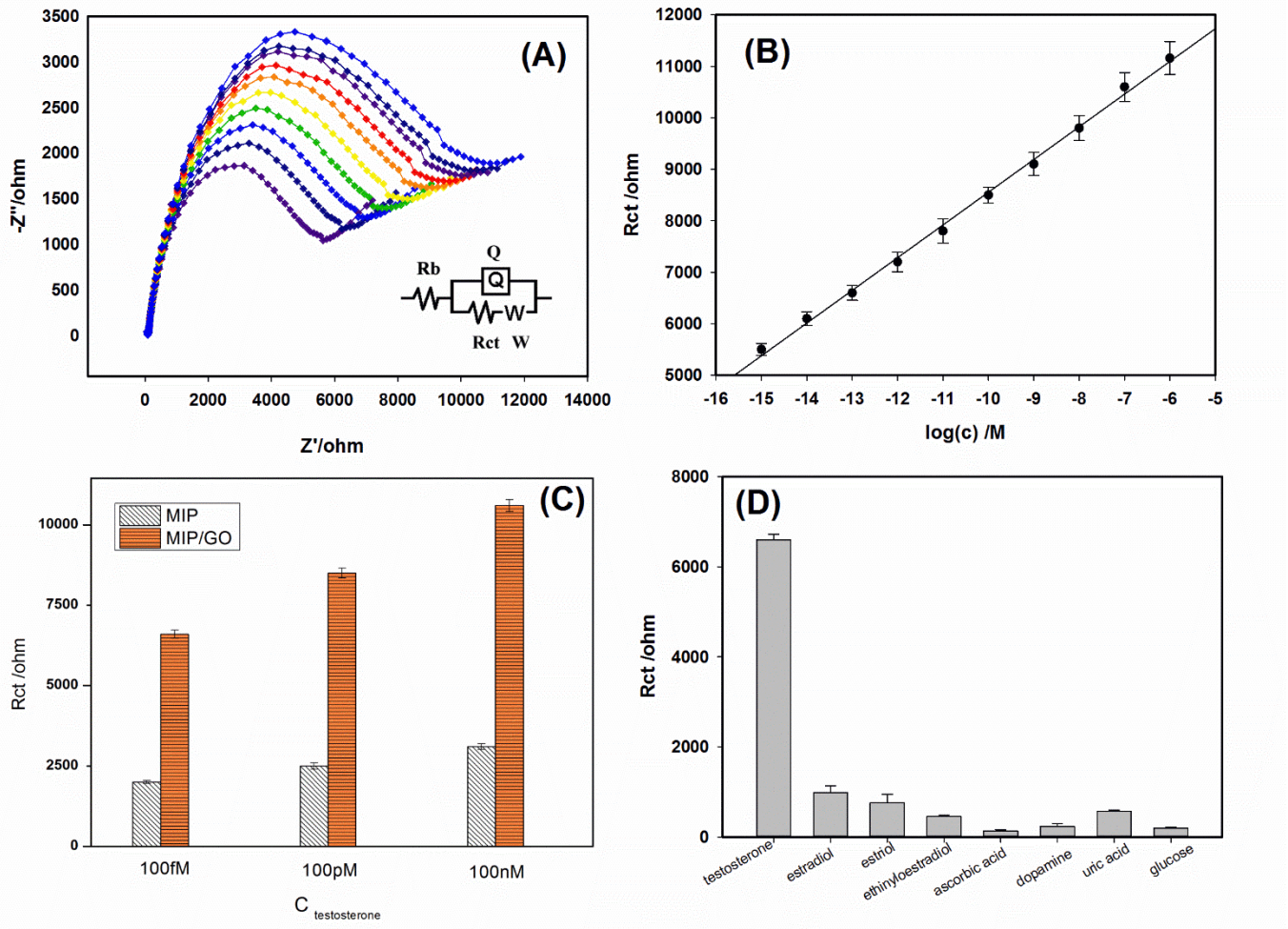


Fig.4



Highlight

- Ultrasensitive determination of testosterone from femtomolar to micromolar levels.
- A nanosized molecularly imprinted polymer film was grafted on the surface of graphene-oxide sheets as a sensitive layer of the sensor.
- Electrochemical impedance spectroscopy (EIS) measurement of the non-redox-active molecule of testosterone.
- A good selectivity over structurally similar steroid hormones, and a long term stability in room temperature.

- Application of the sensor in the determination of testosterone in biological fluids.