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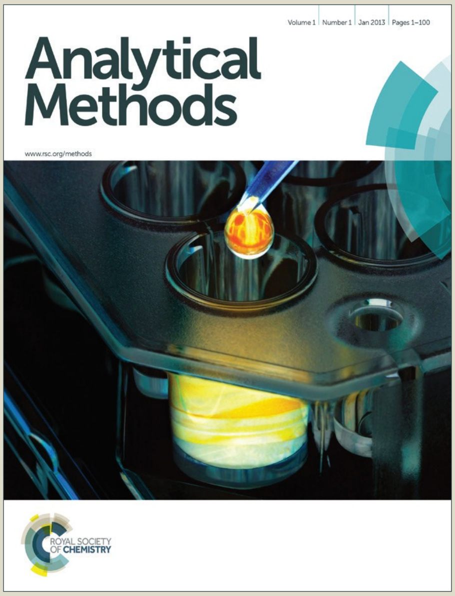

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Electrochemical detection of natural estrogens using graphene/ordered mesoporous carbon modified carbon paste electrode

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Yanqiong Zhu,[a] Xiangjian Liu, [b] and Jianbo Jia [, b]*

A novel electrochemical biosensor based on electrodeposited graphene/ordered mesoporous carbon composite modified carbon paste electrode (GR/OMC/CPE) was prepared for the quantitative determination of natural estrogens by square wave voltammetry (SWV). Electrodeposited GR/OMC composite modified electrode was prepared with CPE by cyclically sweeping in the potential range from −0.8 to +2.4 V at the scan rate of 100 mV s^{-1} in 0.1 mg mL^{-1} OMC and GR, respectively. The morphology of GR/OMC composite was characterized by scanning electron microscopy. The electrochemical behaviors of estrogens at the GR/OMC/CPE were investigated by cyclic voltammetry, and the results showed that GR/OMC/CPE exhibited excellent electrocatalytic activity toward the oxidation of natural estrogens involving estrone, estradiol, and estriol. SWV was used for the quantitative determination of estrogens. Under the optimal conditions, the current response was linearly proportional to the concentration of estrogens in the range from 5.0×10^{-9} to 2.0×10^{-6} mol L^{-1}, with a low detection limit of 2.0×10^{-9} mol L^{-1} (S/N=3). The proposed sensor was successfully applied to analyze estrogens level in female serum samples.

1. Introduction

The essential steroid estrogens, mainly involving estrone (E1), estradiol (E2), and estriol (E3), are important bioactive substances, participating in intracellular communications and influencing the development of sex characteristics [1]. Estrogens not only play crucial roles in the growth and maintenance of the reproductive system, but also affect the functions of many other systems such as skeletal, immune, and neuronal systems. Various techniques, including high-performance liquid chromatography [2-3], fluorescent spectroscopy [4], chromatography-mass spectrometry [5, 6] and immunosorbent assay techniques [7], have been developed for the analysis of estrogens. However, these methods need high-cost instruments, complicated preconcentration and time-consuming steps, limiting applications of these conventional methods. Therefore, it is necessary to explore sensitive, rapid and portable methods to facilitate the detection of estrogens. Nowadays, electrochemical techniques are of particular advantage because of their high sensitivity, a relatively low detection limit, good stability and rapid analysis [8-10]. Consequently, it provides a convenient way to detect the estrogens in complex biological matrices or biofluids.

In recent years, nanoscale materials with high surface area have been widely used for the fabrication of electrochemical sensors to enhance the detection sensitivity [11]. Ordered mesoporous carbon (OMC) is a kind of advanced carbon materials, which merits such as extremely well-ordered pore structure, high specific surface area and excellent electrocatalytic activity, enabling it a promising nanomaterial for electrode modification [12-16]. Nevertheless, the low conductivity of OMC inhibits its application as electrode support material [17, 18]. Some studies have focused on decorating nanomaterials on OMC in order to increase its electrical conductivity and broaden applications [19-20].

Graphene (GR), a novel class of 2D carbon-based nanomaterial with sp^2-bonded carbon atoms, has been the focus of numerous investigations in recent years due to its unique electronic, mechanical, and thermal properties [21]. GR possesses high specific surface area and exhibits excellent electrical conductivity for providing electrical pathways, which makes it a promising candidate for ameliorating the electrical conductivity of OMC and widening the range of applications as electrode materials [22-24]. Furthermore, GR possesses excellent biocompatibility, giving rise to a possibility of GR being used for detection of bioactive substances [25-26].

In this work, we have tried to combine the advantages of both GR and OMC for electrocatalytical oxidation of estrogens. GR and OMC were electrodeposited on the surface of carbon paste electrode (CPE) to fabricate GR/OMC composite modified CPE (GR/OMC/CPE). The designed sensor displayed good performances of rapid detection, low detection limit, and wide linear range for estrogens under the optimal conditions. Furthermore, the

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[a] The Second People's Hospitial of Jingmen, Jingmen 448000, PR China.
[b] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, PR China. E-mail: jbjia@ciac.ac.cn (J. Jia).

presented sensor can be applied to the determination of estrogens in female blood serums with satisfying results.

2. Experimental

2.1 Reagents and apparatus

E1, E2, and E3 were purchased from Sigma (USA). Graphite powder (spectral reagent), paraffin oil, and ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Stock solutions of E1, E2, and E3 (1.0 mmol L^{-1}) were prepared in ethanol. All other chemicals were of analytical grade and double distilled water was used throughout the experiment. 0.1 mol L^{-1} phosphate buffer solutions (PBS) with different pH values adjusted by K_2HPO_4 and KH_2PO_4, were employed as supporting electrolyte.

Electrochemical experiments including cyclic voltametry (CV) and square wave voltammetry (SWV) were carried with a CHI 660D electrochemical workstation (Shanghai CH Instrument Co., China) with a conventional three-electrode system consisting of a GR/OMC/CPE working electrode, a saturated calomel reference electrode (SCE), and a platinum foil counter electrode. Scanning electron microscopy (SEM) images were performed on scanning electron microscope (JSM-6700F, Japan) at 15 kV.

2.2 Synthesis of OMC

OMC was synthesized using template method in which SBA-15 acted as the template and sucrose acted as the carbon source [27-28].

2.3 Electrode preparation and modification

Firstly, graphite powder and paraffin oil were mixed at the ratio of 3:1 (w/w) and ground by pestle in the agate mortar for 20 min. Then, the mixture was firmly packed into a glass tube (i. d. = 2.6 mm). Finally, a copper wire was inserted from another end of the tube. The CPE was polished with a piece of weighing paper and rinsed with double distilled water.

With the aid of ultrasonication, 1.0 mg OMC and 1.0 mg GR were separately dispersed in 10 mL PBS (0.1 mol L^{-1}, pH 7.0). Afterwards, the prepared CPE was put into 0.10 mg mL^{-1} OMC, and swept for 20 segments at the scan rate of 100 mV s^{-1} in the potential range of −0.8—+2.4 V. Then the GR/OMC/CPE was gained via electrodeposition by applying a potential range of −0.8— +2.4 V containing 0.10 mg mL^{-1} GR. Finally, the as-prepared electrodes were rinsed carefully with double distilled water for further use. For comparison, similar procedure was performed to get GR/CPE or OMC/CPE, respectively.

2.4 Electrochemical measurement

SWV were carried out for electrochemical measurements. For SWV, the following instrumental parameters were used: pulse amplitude 25 mV, frequency 40 Hz, and potential step 10 mV. Before the voltammetric determination, the prepared GR/OMC/CPE was immersed in 0.1 mol L^{-1} PBS (pH 7.4) and repeatedly scanned in the potential range from 0.2 to 0.9 V until stable voltammograms were obtained. After that, the GR/OMC/CPE was placed in 0.1 mol L^{-1} PBS (pH 7.4) containing desired concentrations of E2 with 4 min open circuit accumulation under stirring condition, and then kept quiet for 30 s. The voltametric responses were recorded in the potential range of 0.2−0.9 V, and the peak current at 0.54 V was obtained. After each measurement, the GR/OMC/GCE was activated by five cyclic sweeps in the potential range of 0.2−0.9 V in 0.1 mol L^{-1} PBS (pH 7.4) to remove any adsorbates and give a reproducible electrode surface.

3. Results and discussion

3.1 Electrodeposition and SEM of GR/OMC

As is well known, carbon materials, such as carbon nanotube, OMC and graphene, can be deposited on electrode surface by CV under a wide potential window [29-33]. In this work, to prepare GR/OMC/CPE, OMC was firstly electrodeposited on CPE by CV for 20 segments in the potential range of −0.8 ~ 2.4 V at 100 mV s^{-1} in 0.10 mg mL^{-1} OMC solution, and then the OMC/CPE was immersed into 0.10 mg mL^{-1} GR solution under the same potential window for 10 segments to obtain GR/OMC/CPE. The formation of GR and GR/OMC films on CPE was directly confirmed by SEM (Fig. 1). As shown in Fig. 1A, OMC film is made up of linked ordered finger-like carbon flakes. SEM image of GR/OMC composite displays that GR is homogeneously distributed on the surface of OMC with diameters ranging from 10 to 20 nm (Fig. 1B). Accordingly, OMC provide an ideal substrate for GR. GR sheets with nanosized and layered structure could effectively enhance the surface area of electrode. Moreover, GR can facilitate electron transport due to its excellent conductivity. The synergetic effect of OMC and GR is expected to be helpful for the electrocatalytic oxidation of estrogens.

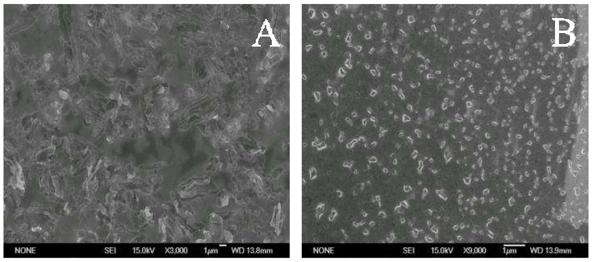

Fig. 1 SEM images of deposited (A) OMC and (B) GR/OMC.

3.2 Electrochemical behaviors of estrogens

The electrochemical performances of E2, E1, and E3 were investigated by CV, as shown in Fig. 2. It can be seen that the electro-oxidation peak currents of estrogens increased successively at different modified electrodes (from a to d). As shown in Fig. 2A, CV of 5.0 μmol L^{-1} E2 in 0.1 mol L^{-1} PBS (pH 7.4) at bare CPE (a) exhibited a small oxidation peak. In contrast, its response currents at GR/CPE (b) and OMC/CPE (c) modified electrodes were about 10 times higher than that of bare CPE (a). A significant increased oxidation peak was observed at GR/OMC/CPE (d) compared to other electrodes, indicating that the electrocatalytic properties of OMC were improved after GR incorporation. This outstanding improvement of current at GR/OMC/CPE is due to synergetic effect of OMC and GR.

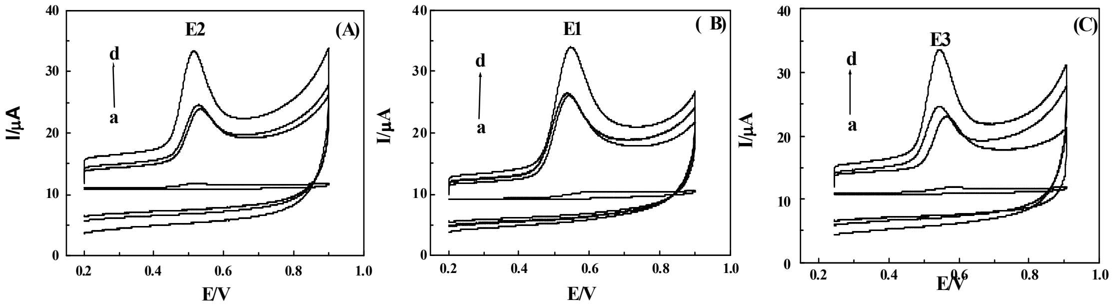

Fig. 2 CVs in 0.1 M PBS (pH 7.4) containing 5.0 μmol L^{-1} (A) E2, (B) E1 and (C) E3 at (a) the bare CPE, (b) OMC/CPE, (c) GR/CPE, and (d) GR/OMC/CPE. The accumulation time is 4 min at open-circuit. Scan rate is 100 mV s^{-1}.

The electrochemical oxidation of 5.0 μmol L^{-1} E2 was investigated at different scan rates (10–200 mV s^{-1}) in 0.1 M PBS (pH 7.4) on GR/OMC/CPE. The oxidation peak currents of E2 increased with the increase of scan rate, and the plot of oxidation peak current against the scan rate shows a linear relationship (Ip (μA) = 0.4862v + 0.4313 with a correlation coefficient of R = 0.998), suggesting that the electro-oxidation of E2 is an adsorption-controlled process.

3.3 Optimization of experimental conditions

In order to further enhance the electrocatalytic performace of GR/OMC/CPE toward estrogens, experimental conditions including the electrodeposition cycles, accumulation time, and pH values were investigated, respectively. Moreover, due to better electrochemical activity, E2 was selected as the representative estrogens for the illustration in the following experiment [33].

3.3.1 Effect of electrodeposition cycles

Electrodeposition cycle is an important factor affecting the morphology and performance of GR/OMC/CPE. In this work, the effect of scanning cycles during electrodeposition process on the catalytic oxidation of 5.0 μmol L^{-1} E2 was investigated by amperometry. Fig. 3 exhibits the effect of electrodeposition cycles of GR on the current response of 5.0 μmol L^{-1} E2. It was found that when cycles shifted from 0–5, the oxidation current increased dramatically. However, when the cycles were more than 5, the peak current decreased slightly. Further increasing the cycles from 10–20, the peak current kept almost constant. This could be explained that the response current of E2 increases with the increasing amount of GR electrocatalyst, but too much GR on the surface of OMC may block the mass and electron transfer on the electrode surface. Therefore, electrodeposition GR cycle of 5 was selected in the following experiment.

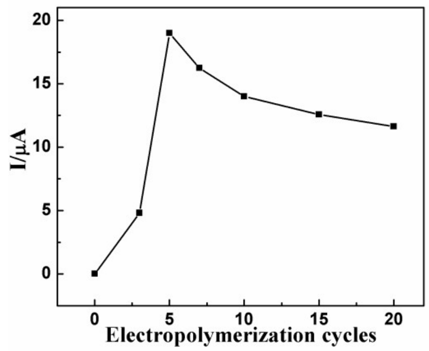

Fig. 3 Effect of electrodeposition cycles on the oxidation of 5.0 μmol L^{-1} E2 at GR/OMC/CPE.

3.3.2 Effect of accumulation time

The relationship between the oxidation peak current and the accumulation time was also studied. As shown in Fig. 4, the response current of E2 increased with the increase of accumulation time from 0 to 4 min and reached maximum at 4 min, when accumulation time exceeded 4 min, the oxidation peak current of E2 decreased. So, accumulation time of 4 min was selected for the determination of E2.

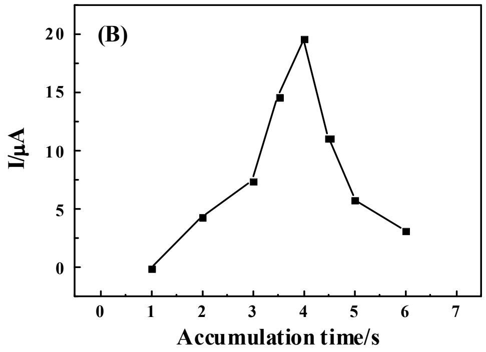

Fig. 4 Effect of accumulation time on the oxidation of 5.0 μmol L^{-1} E2 at GR/OMC/CPE.

3.3.3 Effect of pH values

The effect of pH on the oxidation of 5.0 μmol L^{-1} E2 at GR/OMC/CPE was studied in 0.1 mol L^{-1} PBS in pH range of 4−10, as shown in Fig. 5. The oxidation peak current of E2 at the GR/OMC/CPE increased dramatically from 4.0 to 7.4, and then decreased in the pH range of 7.4−10. Thus, the optimal pH of 7.4 was selected.

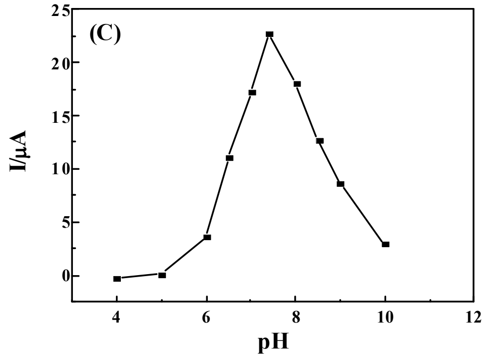

Fig. 5 Effect of pH on the oxidation of 5.0 μmol L^{-1} E2 at GR/OMC/GCE.

3.4 Linearity, detection limit and interferences

Under optimized conditions, SWV was used to monitor the performance of the GR/OMC/CPE in 0.1 mol L^{-1} PBS (pH 7.4) containing E2 with increasing concentrations. The current responses of E2 with different concentrations after 4 min accumulation time were studied, and the results were shown in Fig. 6. The resulting calibration plot is linear over the range from $5.0{\times}10^{-9}$ to $2.0{\times}10^{-6}$ mol L^{-1} for E2, as given in inset of Fig. 6. The calibration curve and correlation coefficient are I_p (μA) = 2.730 + 7.444C (μmol L^{-1}) and 0.9905, respectively. Based on three times the background noise, the limits of detection was $2.0{\times}10^{-9}$ mol L^{-1}.

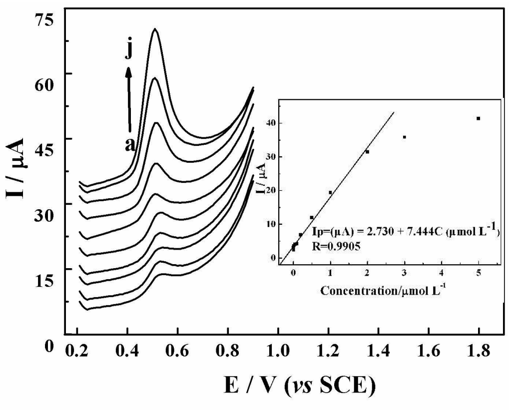

Fig. 6 SWVs of GR/OMC/GCE in 0.1 mol L^{-1} PBS (pH 7.4) solution containing different concentrations of E2 (a to i: $5.0{\times}10^{-9}$ to $2.0{\times}10^{-4}$ mol L^{-1}) after accumulation 4 min; inset is the calibration curve for the determination of E2.

The interference study aimed to investigate the effects of possible existing metal ions and biological molecules on the determination of E2. The interference experiments were performed in 0.1 mol L^{-1} PBS (pH 7.4) by comparing the response current of 1.0 μmol L^{-1} E2 with that of different concentrations of each interfering substance. The tolerance limit was taken as the maximum concentration of the foreign substances causing an approximately ± 5% relative error in the determination. The results showed that 500-fold of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, Ac^-, glucose, and leucine, 100-fold of dopamine, uric acid, ascorbic acid, epinephrine, leucine, alanine, phenylalanine, and cysteine had no significant interference. Nevertheless, tyrosine and tryptophan have serious influence on the determination for their similar molecular structure with estrogens, which may cause false positive signal. To eliminate their interference, solid-phase microextraction technique can be used as an effective sample preprocessing method before measurement in real samples.

3.5 Analytical applications

In order to investigate the reliability of the proposed sensor in real samples, GR/OMC/CPE was utilized to detect E2 concentration in female serum samples. The results are listed in Table 1. It can be seen that the average recovery of E2 was between 95.5% and 102.1%, indicating the feasibility of the presented method.

Table 1 Determination of E2 concentration in the blood serum samples.

Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery (%)	R.S.D. (%)
0.10	0.0955	95.50	4.5
0.10	0.0992	99.29	0.8
0.10	0.1021	102.10	2.1

4. Conclusions

In this work, we report a novel electrochemical biosensor based on electrodeposited GR/OMC composite modified carbon paste electrode for the quantitative determination of natural estrogens by SWV. The prepared GR/OMC/CPE exhibited great electrocatalytic activity towards the oxidation of natural estrogens. The proposed sensor is easy to produce, and promising for determination of natural estrogens in real samples.

Acknowledgements

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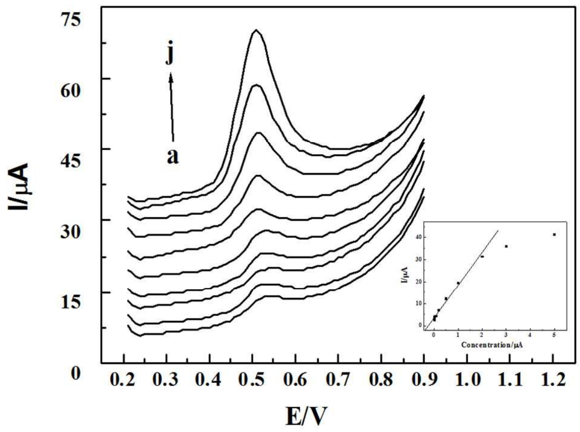
Graphical Abstract



SWVs of GR/OMC/GCE in 0.1 M PBS (pH 7.4) solution containing different concentrations of estradiol.