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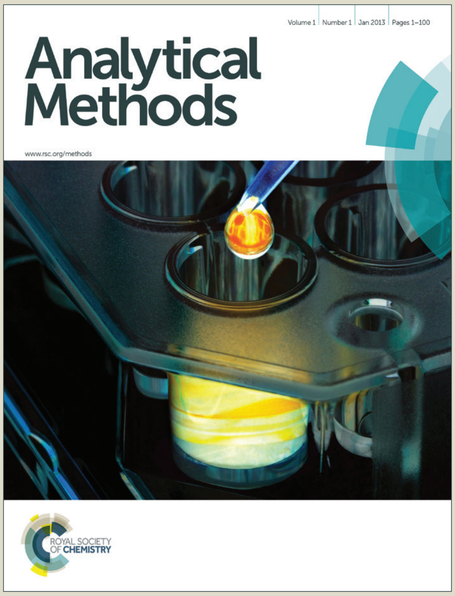

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Amplified electrochemiluminescence of lucigenin triggered by electrochemically reduced graphene oxide and its sensitively detection for bisphenol A

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Abstract

An amplified and stable electrochemiluminescent signal of lucigenin on electrochemically reduced graphene oxide contained sensing platform was observed. Herein, chitosan was used as the excellent dispersant, which in favor of the better dispersion of graphene oxide in solution and the better electrochemical reduction of graphene oxide. And the electrochemically reduced graphene oxide owned the outstanding conductivity which was propitious to the transfer of electrons, leading to the enhancement of electrochemiluminescent signals of lucigenin. Additionally, bisphenol A, as a classical foodborne pollutant, was firstly detected due to its strong inhibition phenomenon to the electrochemiluminescent response of lucigenin. Then we studied the quenched electrochemiluminescent system and the possible mechanism of the platform in detail. Under optimum conditions, the proposed electrochemiluminescent sensor exhibited a linear response range from 1.0×10^{-9} mol L^{-1} to 1.0×10^{-4} mol L^{-1} with a low detection limit of 3.0×10^{-10} mol L^{-1} for bisphenol A, which might find promising applications in developing a new type of biosensor.

Keywords: electrochemiluminescent, lucigenin, electrochemically reduced graphene oxide, chitosan, bisphenol A

1 Introduction

Electrochemiluminescence, also called as electrogenerated chemiluminescence, means that electric energy is converted into radioactive energy at the electrode surface through an applied potential.[1,2] Luminescent signals can be obtained from the excited states of an electrochemiluminescent (ECL) luminophore generated at electrode surfaces during the electrochemical reaction.[1,2] In recent years, there has been a steady growth of focus in electrochemiluminescence which is considered as an important and valuable detection method in analytical chemistry due to its high sensitivity, high selectivity and good reproducibility.[3,4] Furthermore, ECL sensors not only retain the advantages of chemiluminescence (CL) sensor, such as the excellent sensitivity and a wide dynamic concentration response range, but also own some additional advantages over the CL sensors. [3,4] Consequently, many researches had been done universally on the three ECL compounds, which include lucigenin (N,N-dimethyl- 9,9-biacridinium dinitrate), luminol and ruthenium complexes, particularly tris(2,2’-bipyridine) ruthenium(II) ($Ru(bpy)_3^{2+}$),[1,5] in which we found that, compared with the ECL regents of luminol and $Ru(bpy)_3^{2+}$, there were only a few literatures about ECL of lucigenin in aqueous media and analytical applications owe to the bad reproducibility. Takeo Ohsaka and Takeyoshi Okajima discovered that the sensitivity and reproducibility of ECL of lucigenin can be improved when doing some researches on the modified electrode with self-assembled monolayers and of solutions with surfactants.[6] Therefore, it is of great importance for extending analytical applications of lucigenin to find a new method for solving the problem of insoluble electrochemical luminescence absorption on the surface of electrode.

Tremendous attention has been paid to graphene together with its various derivatives,[7] such as reduced graphene oxide (RGO),[8,9] graphene nanoribbon[10] and nitrogen-doped graphene[11] in recent years because of their excellent electrical conductivity. In especial, RGO , a material of two-dimensional material, is robust and popular owe to its good dispersibility, convenient chemical modification, good mechanical and thermal properties, high surface area, easy functionalization and good biocompatibility.[12] These interesting properties make RGO a promising material for extraordinary applications in the fields such as batteries[13], catalysis[14], nanoelectronics[15] and supercapacitors[16]. Various methods including micromechanical cleavage[17], solvothermal synthesis[18], epitaxial growth[19,20] and liquid-phase exfoliation[21-23] have been proposed to prepare RGO. However, the widespread applications of RGO are limited by high temperature, special equipment and conditions, and tedious procedures during its preparation. Recently, there have been reported that graphene sheets can be prepared through GO as a precursor in a bulk route by non-composite conversion of GO into “chemically reduced” graphene oxide making use of thermal treatment[24] or chemical reducing agents[25], which may be cheap and of high synthesized efficiency but poisonous and intricacy. More recently, Srinivasan Sampath have successfully combined raman spectroscopy with electrochemistry to accomplish electrochemical reduction of GO to RGO, and realized an in situ raman spectroelectrochemical study.[26] Shaojun Dong have actualized controlled synthesis of large-area and patterned electrochemically reduced graphene oxide (ERGO) films.[27] Direct electrochemical reduction of single-layer GO and subsequent functionalization with glucose oxidase have been achieved by Hua Zhang and his coworkers,[28] which could obtain more highly conducting graphene that possessed better biocompatibility on electrode materials. It is therefore of our interest to develop an efficient, facile, environment friendly and low-cost electrochemical method to produce RGO films. Herein, we dispersed GO in a certain concentration chitosan (CS) solution and cast the GO-CS solution on the bare gold electrode, then reduced the modified electrode at certain potential. Not only this modified electrode amplified the ECL emission of lucigenin, but also evidently improved the stability of the ECL response of lucigenin. Herein, CS was used to disperse GO and provide an excellent environment to construct sensors due to its attractive properties, including excellent high permeability toward water, film-forming ability, biocompatibility, good adhesion, nontoxicity, biocompatibility, high mechanical strength and a susceptibility to chemical modifications.[29-32] Besides, an ECL inhibition method for the determination of trace bisphenol A (BPA) has been established based on the strong inhibition phenomenon of BPA to the ECL values of lucigenin. Under the optimum conditions, the sensor exhibited high sensitivity, good stability and specificity for the quantitative detection of BPA, which may have promising applications for the fabrication of ECL array sensors.

2 Experimental

2.1 Reagents

Chitosan (CS), potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide

($K_4Fe(CN)_6$), potassium chloride (KCl), hydrochloric acid (HCl), acetic acid (CH_3COOH) and sodium acetate (CH_3COONa) were purchased from Chemicals Co., Ltd. (Shanghai, China). 0.1 mol L^{-1} acetate buffer solutions at various pH values were prepared from the stock solutions of 0.1 mol L^{-1} CH_3COOH and CH_3COONa solution. Lucigenin was obtained from Fluka Chemie AG. 1.0×10^{-3} mol L^{-1} stock solution of lucigenin was prepared by dissolving lucigenin in redistilled water. All reagents were used without further purification. All aqueous solutions and the water for rinsing were prepared from Ultrapure water from a Milli-Q plus system (Millipore Co., >18 MX cm^{-1}).

2.2 Apparatus

A homemade ECL system including a BPCL Ultra-Weak Chemiluminescence Analyzer (Institute of Biophysics, Chinese Academy of Sciences) for detecting light emission and an Electrochemistry Working Station (Chenhua 660A, Shanghai, China) was used to detect ECL intensity versus potential. All experiments were carried out with a conventional three-electrode system in which a gold electrode (GE) (2 mm diameter), KCl-saturated silver–silver chloride (Ag/AgCl) and a platinum wire acted as the working, reference and counter electrodes, respectively. All potentials reported here refer to the Ag/AgCl (sat. KCl) reference electrode. X-ray photoelectron spectroscopy (XPS) measurements were carried out with ESCALAB 250 Å 1314. Transmission Electron Microscope (TEM) measurements were executed with JEM-2100F, Janpan. The solution pH measurements were carried out on a pHS-3C pH meter equipped with a composite pH glass electrode (Shanghai Dapu Instr. Inc., China), after careful pH-calibration procedures.

2.3 Preparation of GO-CS colloidal suspensions

The preparation of GO was based on Hummers' method described in the literature.[33] In a typical procedure, 0.2 g of graphite powder was added into 3.5 mL H_2SO_4 and stirred for 2 h, in the same time adding 0.6 g $KMnO_4$ slowly to maintain the temperature of the solution below 293 K under constant stirring. The resulting mixture was then stirred at 308 K for 30 min then 15 mL H_2O and 2 mL 30% H_2O_2 solutions were added to terminate the oxidation. The mixture was then washed with 5% HCl aqueous solution repeatedly until the pH became neutral. 16 mL water was added into the resulting precipitate and sonicated well to obtain GO. The brown dispersion was centrifuged at 4000 rpm for 2 h to remove any unexfoliated GO. Then 8 mg mL^{-1} GO suspension was prepared by dissolving 4 mg GO in 500 μL deionized water and vibrating for 5 min at room temperature until obtaining a homogeneous GO solution. A 1.0% CS solution was prepared by dissolving 1.0 g CS solid in 100 mL of 1.0 % acetic acid solution and stirring for several hours at room temperature until reaching a homogeneous CS solution, and the CS solution was stored in refrigerator when it was not in use. A certain proportion GO-CS was prepared by mixing certain volume 8 mg mL^{-1} GO suspensions with certain volume 1.0% chitosan solution, and then vibrating and sonicating until obtaining a homogeneous solution.

2.4 Preparation and reduction of GO-CS modified gold electrode

The mechanism for fabrication of ECL sensor was shown in Schem. 1A. Before modification, the bare gold electrode (GE) was firstly polished with 0.05 μm alumina slurry, and next sonicated in deionized water to remove the trace remainder, then immersed in freshly prepared Piranha solution (30% H_2O_2 and concentrated H_2SO_4, 3:1 (v/v)) for 10 min, and finally electrochemically pre-treated by cyclic potential scanning between 1.4 V and −0.2 V in 0.1 mol L^{-1} H_2SO_4 until cyclic voltammogram of clean gold electrode was obtained. During modification, 3 μL prepared GO-CS colloidal suspension was casted on the pre-treated gold electrode, dried under infrared lamp and cooled to room temperature. Then, the GO-CS modified gold electrode was reduced in CH_3COOH-CH_3COONa (pH 5.0) buffer with 0.1 mol L^{-1} KCl solution at -1.3 V for 400 s.

3 Results and discussion

3.1 The morphology of the reduced modified electrode

The morphological and structural characterization of GO was performed by TEM. As shown in Fig.1A, we can clearly found that the GO possessed transparent and similar to silk veil large layered structure. The XPS of the GO-CS and ERGO-CS modified gold electrodes were exhibited in Fig. 1B and Fig. 1C, respectively. The peak at 284.5, 286.4 and 288.2 eV, which stand for C-C, C-O and C=O bands in graphene-based materials, respectively. By comparing Fig. 1B with 1C, after electrochemical reduction, we can know that the peak intensity of functional groups that contain the element of oxygen decreased tremendously while that of carbon-carbon bond increased. The result confirmed that the effectiveness of the electrochemical reduction and the GO can be reduced through the electrochemical method in the CS modified layer.

3.2 Electrochemical behavior of various modified electrodes

As can be seen from Fig. 2A, there were cyclic voltammograms (CVs) of ERGO-CS/GE (a), GO-CS/GE (c) and GE (b). A classic pair of redox peaks was obtained at the bare gold electrode. Whereas, the introduced of GO/CS composite film significantly dwindled the oxidation/reduction of $Fe(CN)_6^{3-/4-}$ on the electrode, which due to not only the GO as the insulator impeded the transfer of electron in the electrode surface, but also the electrostatic repulsion between GO/CS composite and electrochemical probes. It is especially noteworthy that we could find the value of peak current of reduced modified electrode obtained at -1.3 V for 400 s was bigger than that of bare gold electrode. This is mainly due to the electrochemical reduction of ERGO possessed high conductivity, which would lead to the electron transfer in electrode surface and the reduction of negative charge in ERGO/CS membrane lessened the electrostatic repulsion to $Fe(CN)_6^{3-}$. The result was well consistent with the phenomenon in early reports, such as, Navarro observed a three-order increase in conductivity when GO was chemically reduced using hydrazine to RGO, and Hua Zhang[28] found the redox peak of RGO-CS modified gold electrode in $[Fe(CN)_6]^{3-/4-}$ was almost the same as that of bare GCE, which also indicated that the conductivity of the electrochemically RGO was as good as that of GCE.

Fig. 2B showed the CVs of the modified electrode in $K_3[Fe(CN)_6]$ solution with various potential scan rates .The relationship redox peak current of $[Fe(CN)_6]^{3-/4-}$ and the square root of scan rate assess the properties of electrode, described by following Randles–Sevcik equation[34]:

$i_p=(2.69\times105)n^{3/2}AD^{1/2}C_0v^{1/2}$

In Fig. 2B inset, we can conclude that the anodic and cathodic peak currents varied linearly with the square root of the scan rate, suggesting that the reaction was a diffuse-controlled process.

The Randles circuit was selected to obtain the impedance data. As we all know, the resistance to charge transfer (R_{ct}) and the diffusion impedance (W) were both in parallel to the interfacial capacitance (C_{dl}). The diameter of the semicircle changed corresponding to the interfacial electron-transfer resistance (R_{ct}). From Fig. 2C, we could easily find that the diameter of the semicircle of bare gold electrode was the smallest and that of ERGO-CS modified electrode was much smaller than the GO-CS modified electrode. We can learn from the phenomenon that the conductivity of reduced electrode was better than the unreduced one and that ERGO-CS composite film could function as an efficient electron conducting tunnel between the electrode and electrolyte.

3.3 ECL characteristics of various modified electrodes

We could see from the Fig. 2D that the ECL values at the bare gold electrode and GO-CS/GE were very low. However, the ECL values of ERGO-CS/GE increased to 4500, almost 9 folds compared with that of GO-CS/GE, demonstrating that ERGO-CS/GE had better conductivity. In addition, the electrochemiluminescence of lucigenin on ERGO-CS/GE occurred at a more positive initial voltage, indicating the ECL process can be carried out faster and easier. From the electrochemical behavior characterization of different modified films, we have achieved that the electrochemical reduction of GO generated ERGO has good conductivity, it could promote the transfer rate of electron in ECL process which lead to faster and stronger ECL intensity. Simultaneously, negatively charged chitosan was propitious to positively charged lucigenin absorbed to electrode surface by electrostatic attraction, which lead to the ECL intensity enhancement of lucigenin in ERGO-CS composite film modified gold electrode.

The ECL mechanism of lucigenin at −0.6 V have been studied and could be intuitively seen in Scheme 1B. Primarily, electrons were transferred from electrode to the lucigenin and O_2 through ERGO-CS, which leaded to the generation of Luc^{+} and $O_2^{\cdot -}$. The cathodic ECL herein was originated from the formation of excited-state NMA^{*} via the transformation of the dioxetane which generated by the reaction of $Luc^{\bullet +}$ and $O_2^{\bullet -}$.[35-39] The corresponding ECL mechanism could be formulated as follows:

$$Luc^{2+} + e^{-} \longrightarrow Luc^{\bullet +} \qquad (1)$$

$$O_2 + e^{-} \longrightarrow O_2^{\bullet -} \qquad (2)$$

$$Luc^{\bullet +} + O_2^{\bullet -} \longrightarrow \text{dioxetane} \longrightarrow NMA + NMA^{*} \qquad (3)$$

$$NMA^{*} \longrightarrow NMA + h\nu \qquad (4)$$

3.4 Influence factors on ECL emission on ERGO-CS/GE

The parameters of the modified electrode affected not only the ECL intensity but also the reproducibility of proposed ECL method. Thus we chose some parameters to investigate in order to get the best analytical performance.

3.4.1 Effect of GO concentration

From Fig. 3A we could find the ECL intensity first increased and then decreased with the increase in the concentrations of GO, and the maximum response was obtained at the concentration of 1.5 mg mL^{-1}, which was attribute to that EGRO obtained by the process of electrochemical reduce the GO was helpful for the electron transfer. Besides, the increased surface area of electrode supported substance transportation and made contribution to the enhanced ECL intensity. However, when the concentration was higher than 1.5 mg mL^{-1}, the thicker modified membrane hindered the mass transfer of reactants resulting in the decrease of ECL intensity,[40] therefore 1.5 mg mL^{-1} GO was selected as the optimal concentration for this electrochemical sensor.

3.4.2 Reduction potential effect

As shown in Fig. 3B, only weak ECL signals could be found when the reduction potential was lower than -1.0 V maybe because of the trace amount of the ERGO. Moreover, we found that in certain range, the higher the reduction potential, the higher the ECL intensity, and then the ECL intensity will keep at a steady value after -1.3 V, suggesting that the GO in the composite film could be almost reduced at -1.3 V.

3.4.3 Reduction time dependence

The reduction time influenced the ECL signals obviously. When the time was less than 400 s, the ECL intensity increased with the reduction time and then reached the largest at 400 s. More than 400 s, the ECL value decreased quickly, as we can see in Fig. 3C. This may be due to that the electrode surface performance could cluster sharply， which was harmful for the electron delivering, and the construction of CS would be violated when reduction time was longer than 400 s at the optimal potential.

3.4.4 The influence of dispensed volume

As can be seen from Fig. 3D, the highest ECL intensity could be obtained when the dispensed volume was 3 μL. The possible reason was that too much modified volume was unfavorable for the ECL behavior and resulted in the increase of non-conductive GO resulting in weaker electron conduction and the thick membrane would hinder the mass transfer of reactants.[36]

3.4.5 CS concentration dependence

From the picture (Fig. 3E) we can know that the concentration of CS used for modified gold electrode had great influence on the ECL intensity. Firstly, the possible reason could be that the higher the concentration of the CS, the better the dispersion of GO in solution, and the easier for solution to form a membrane, which was in favor of mass transfer. Secondly, negative charged CS was propitious to the accumulation of lucigenin on the surface of electrode, which could also lead to the increase of ECL intensity. We tried different concentrations of CS and found that when the value was 0.3%, the ECL was strongest, so 0.3% CS was selected for subsequent studies.

3.5 The linearity of lucigenin on the ERGO-CS/GE and GE

Fig. 4A revealed that the linear range of lucigenin on the ERGO-CS/GE is wider than that on bare gold electrode, ranging from 1.0×10^{-7} mol L^{-1} to 1.0×10^{-4} mol L^{-1}, and the regression equation on the ERGO-CS/GE was lg(ECL) = 0.5174 lgC + 6.0585 (R^2 = 0.994), where ECL is the ECL intensity; C is the concentration of lucigenin, while on the bare gold electrode, only ranging from 5.0×10^{-6} mol L^{-1} to 1.0×10^{-4} mol L^{-1}, and the regression equation was lg(ECL) = 1.2918 lgC + 8.6151 ($R^2 = 0.997$), where the ECL and C stay just the same as before.

3.6 Stability and reproducibility of the modified electrode

Fig. 4B showed the ECL intensity of ERGO-CS/GE in the optimal parameters. We knew from the figure that the ECL intensity remained almost the same under the optimum conditions scanning for 10 cycles and the relative standard deviation (RSD) of the ECL intensity was only 5%, which indicated that the prepared sensor possessed good stability and showed potential for practical applications. Reproducibility of the modified electrode was investigated in 20 μmol L^{-1} lucigenin for three different ERGO-CS modified electrodes. Three measurements resulted in a relative standard derivation of 3.4%, showing good reproducibility of the fabrication protocol. Besides, when the electrode was not in use, it was stored in the shade at room temperature and measured every week. No obvious decrease in the photocurrent response to lucigenin was observed after 2 weeks, and 93% of the initial photocurrent response was maintained after one month. This was attribute to the improvement of hydrophily of the membrane on the modified electrode, which played a role in avoiding the absorption of resultant of lucigenin reaction which is of hydrophobicity. We can take advantage of the property that the ECL sensor was very steady and the chemical property of ERGO was stable in the following studies.

3.7 Practical Detection

BPA, an estrogenic endocrine-disrupting chemical, is used in the lacquer lining of metal food cans and water pipes, food packing and dental sealants, which has been attracting more and more attention due to a large body of assured reports pointed out that BPA can induce a variety of adverse effects including abnormal weight gain, prostate cancer and insulin resistance in human. Recently, analysis of BPA has mainly been accomplished by high performance liquid chromatography-mass spectrometry,[41] gas chromatography-mass spectrometry,[42] and liquid chromatography,[43] with various detectors. Nonetheless, they were low sensitivity, time consuming, and needing troublesome sample preparation, highly qualified technicians and sophisticated equipments.[44] Consequently, an eco-friendliness, fast and sensitive method based on the strong inhibition phenomenon of BPA to the ECL values of lucigenin to monitoring the trace level BPA in biological and environmental samples.

3.7.1 Determination mechanism

Scheme 1C demonstrated the possible mechanism about how the ECL signal of lucigenin was quenched by BPA. The possible quenching mechanism between lucigenin and BPA was similar to that between lucigenin and carbolic acid[37] as well as aniline[45]. Chemical compound that has electron-donating group, especially ·OH, $\cdot NH_2$, would restrain the ECL of lucigenin. While compounds that have intense reduction ability, such as phenol compounds can act as good antioxygen which could strongly quench the ECL of lucigenin as well. From Fig.1 we could clearly know that the BPA possess two ·OH, BPA may be oxidized when $O_2\cdot^-$ encountered the BPA and the chance of $O_2\cdot^-$ coming across the $Luc\cdot^+$ would be reduced simultaneity, as a result, the ECL values decreased after BPA being injected into lucigenin solution.

3.7.2 Condition optimization in the BPA detection

We could see that the ECL intensity was strongest when the pH value was 5.0 as Fig. 5A showed. This may be ascribed to that protonation involved in the electrochemical reduction process when at lower pH values,[37] however, when the pH value was lower than 5.0, the ECL intensity decreased, which could be attributed to that the CS and GO could dissolve in such a buffer as we all know that the CS could dissolve in the acid solution. Therefore, the ECL detection was performed in pH 5.0 buffer solutions.

Besides, the effect of the KCl concentration in buffer solution (5.0) on the ECL intensity of the sensor was investigated, which revealed clearly that the concentration of KCl in buffer solution tremendously influenced the ECL intensity, the lower the lg (concentration of KCl), the stronger the ECL intensity in certain range. As clearly depicted in Fig. 5B, when the concentration of KCl was higher than 0.01mol L^{-1}, the background signal increased greatly.[37] The maximum ECL was obtained in 1×10^{-4} mol L^{-1} according to the figure. And when the concentration of KCl was lower than 1×10^{-4} mol L^{-1}, the tendency changed inconspicuously, so 1×10^{-4} mol L^{-1} was the optimum concentration of KCl.

Finally, the concentration of lucigenin also produced a great influence on the ECL intensity. According to the study before, we know that, first, when the concentration was higher than 1×10^{-4} mol L^{-1}, the ECL background signal of lucigenin was quite high and it weakened the ECL intensity, which was harmful for achieving higher sensitivity. Second, higher concentration of lucigenin would produce more reductive product, which was of low solubility and would adsorb on the surface of modified electrode. Third, the lucigenin was expensive. Considering all mentioned above, we selected 1×10^{-4} mol L^{-1} as the optimal concentration of lucigenin.

3.7.3 Calibration Curve, Linear Range, and Detection Limit.

As shown in Fig. 5C, on conditions of the optimum, the ECL intensity was inversely proportional to the concentration of BPA, ranging from 1.0×10^{-9} to 1.0×10^{-4} mol L^{-1}, and the regression equation was lg(ECL) = -423.94 lgC -944.2 ($R^2 = 0.9975$), where ECL is the ECL intensity, C is the concentration of BPA. The relative standard deviation for 1.0×10^{-9} mol L^{-1} BPA was 3.29% (n = 5). The detection limit (defined as the concentration that could be detected at the signal-to-noise ratio of 3) was 3×10^{-10} mol L^{-1}.

3.7.4 Interference

The practical utility of a sensor often depends upon its selectivity, thus, the influence of some potential coexisting ions and other compounds in biological sample were investigated. The responses of 500-fold for Na^+, SO_4^{2-}, NO_3^-, 5-fold for maltose, citric acid, sucrose, and 3-fold for uric acid and ascorbic acid had no evident influence on the determination of BPA, suggesting the novel ECL sensor had high selectivity toward BPA from other coexisting species.

4. Conclusion

A simple, low cost and high efficiency electrochemical method was used to transform GO into ERGO successfully. And a composite film made of GO and CS was used to fabricate modified gold electrode, and then conventional cyclic voltammetry was posed on the modified electrode to produce ERGO-CS composite film modified electrode. The new ECL sensor we proposed displayed supersensitive and selective detection of BPA through a lucigenin ECL signal amplification mechanism. Due to ERGO possessed better conductivity than GO and it played a great role in this platform, thereby, it could greatly avoid the adsorption of electrochemical reaction products on the electrode and greatly improve the sensitivity of detecting BPA. Furthermore, this material could be also used in other areas, such as electrochemistry, immunoassay and so on. Accordingly, this text present an important strategy to design ECL sensors and expand their applications of ultratrace amount environmental sensing.

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Figure captions

Scheme 1 Schematic representation of (A) the preparation of the ERGO-CS modified electrode, (B) the mechanism of ECL emission of lucigenin and (C) the possible quenched ECL mechanism by BPA.

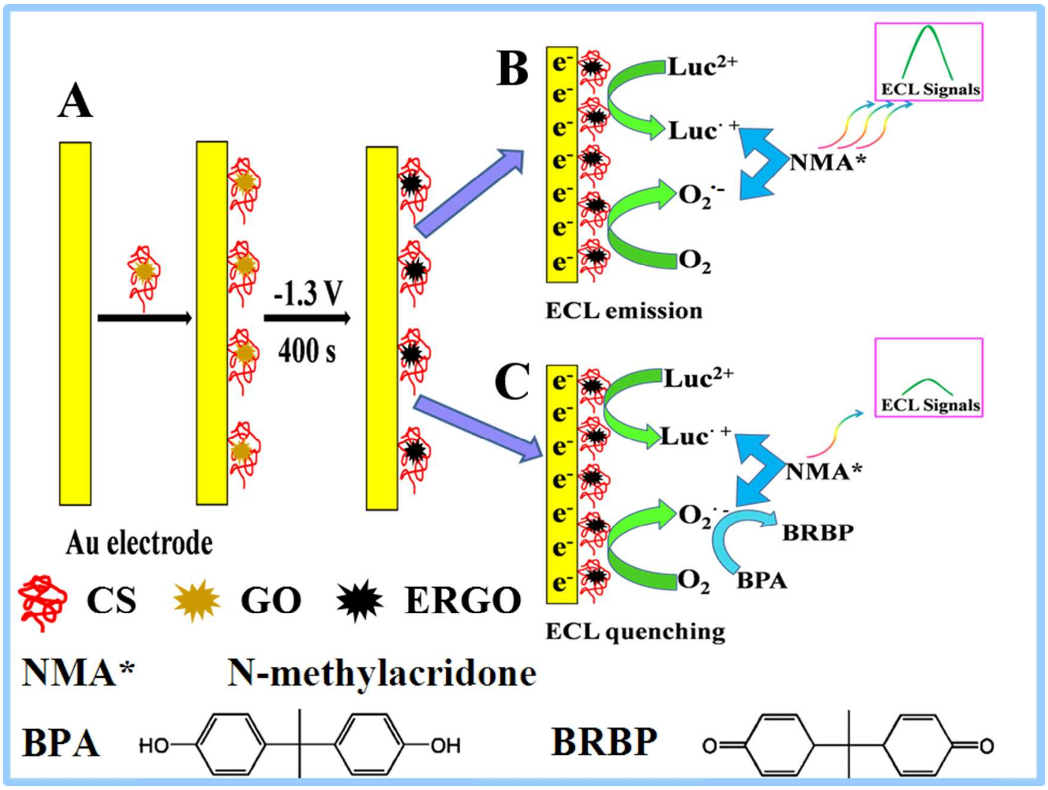
Figure 1 (A) TEM image of GO in CS solution, XPS images of the GO-CS (B) and REGO-CS (C) modified electrodes.

Figure 2 (A) Cyclic voltammograms of REGO-CS modified electrode (a), bare gold electrode (b), and the GO-CS modified gold electrode (c) in 5 mmol L^{-1} $[Fe(CN)_6]^{3-}$ solution containing 0.3 mol L^{-1} KCl, scan rate：0.1 V/s; (B) Cyclic voltammograms of reduced GO-CS modified electrode in 5mM $K_3[Fe(CN)_6]$ solution containing 0.3 M KCl with different scan rates and the linear relationship between the peak currents and the square root of the scan rates (the inset in B), scan rate (a-k) is from 500 to 10 mV/s; (C) Nyquist plots of (a) GO-CS modified, (b) ERGO-CS modified, and (c) bare gold electrodes in 5 mmol L^{-1} $[Fe(CN)_6]^{3-}$ solution containing 0.01 mol L^{-1} KCl, frequency range: 0.1 to 10 KHz; (D) The ECL of different electrodes in 0.1 mmol L^{-1} KCl containing 20 μmol L^{-1} lucigenin with the scan rate of 0.1 V/s, (a) ERGO-CS modified gold electrode, (b) bare gold electrode, (c) GO-CS modified gold electrode.

Figure 3 Effects of (A) the concentration of GO, (B) reduction potential, (C) reduction time, (D) dispend volume, and (E) the concentration of CS on the ECL intensity of ERGO-CS modified electrode in 0.01 mol L^{-1} KCl containing 20 μmol L^{-1} lucigenin.

Figure 4 (A) Calibration plots of lucigenin at (a) ERGO-CS modified gold electrode and (b) bare gold electrode, condition: KCl 0.1 mmol L^{-1} KCl, Potential window:-1.5-0 V, scan rate: 0.1 V/s; (B) ECL signals for duplicate measurements of lucigenin at the concentration of 20 μmol L^{-1} in 0.1 mmol L^{-1} KCl on ERGO-CS modified electrode with the scan rate of 0.1V/s.

Figure 5 Effects of pH (A) and KCl concentration (B) on the lucigenin ECL intensity. Condition: GO 1.5mg/ml with 0.3% CS, Reduction potential -1.3 V, Reduction time 400s, Dispensed volume 3 μL ; (C) ECL responses of the proposed sensor to different concentration (inter to outer is from 1×10^{-4} to 1×10^{-9} mol L^{-1}) of BPA ,and linear plot for BPA determination (the inset in (C)).


A
Au electrode
-1.3 V
400 s
B
e⁻
Luc2+
Luc·+
NMA*
O2·-
O2
ECL Signals
ECL emission
C
Luc2+
Luc·+
NMA*
O2·-
O2
BRBP
BPA
ECL Signals
ECL quenching
CS
GO
ERGO
NMA* N-methylacridone
BPA
HO
OH
BRBP
O
O

Scheme 1

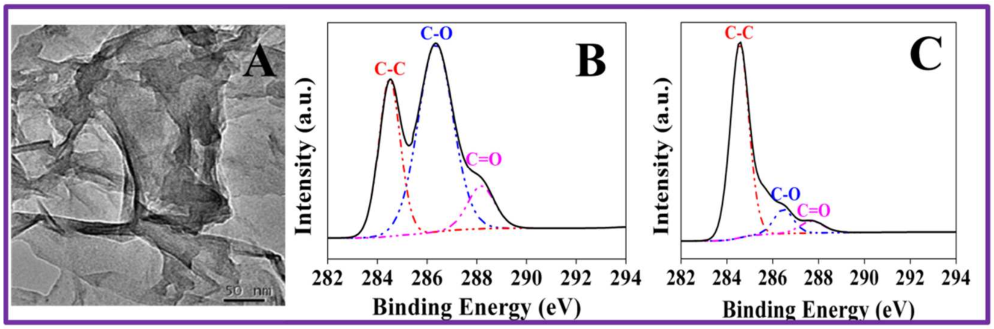

A
50 nm
B
C-C
C-O
C=O
Intensity (a.u.)
282 284 286 288 290 292 294
Binding Energy (eV)
C
C-C
C-O
C=O
Intensity (a.u.)
282 284 286 288 290 292 294
Binding Energy (eV)

Figure 1

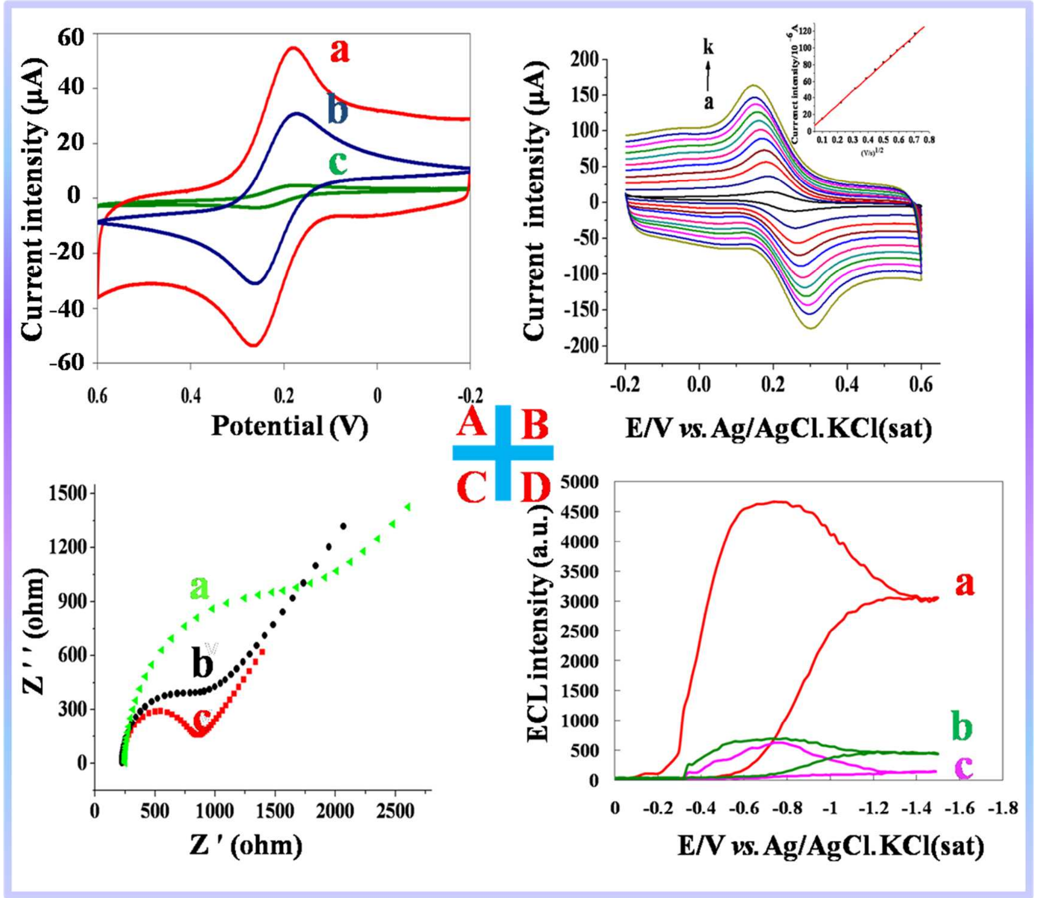

A
B
C
D
Current intensity (μA)
60
40
20
0
-20
-40
-60
0.6
0.4
0.2
0
-0.2
Potential (V)
a
b
c
Current intensity (μA)
200
150
100
50
0
-50
-100
-150
-200
-0.2
0.0
0.2
0.4
0.6
E/V vs. Ag/AgCl.KCl(sat)
k
a
Current intensity/10⁻⁶A
120
100
80
60
40
20
0
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8
(V/s)^1/2
Z'' (ohm)
1500
1200
900
600
300
0
0
500
1000
1500
2000
2500
Z' (ohm)
a
b
c
ECL intensity (a.u.)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
-1.6
-1.8
E/V vs. Ag/AgCl.KCl(sat)
a
b
c

Figure 2

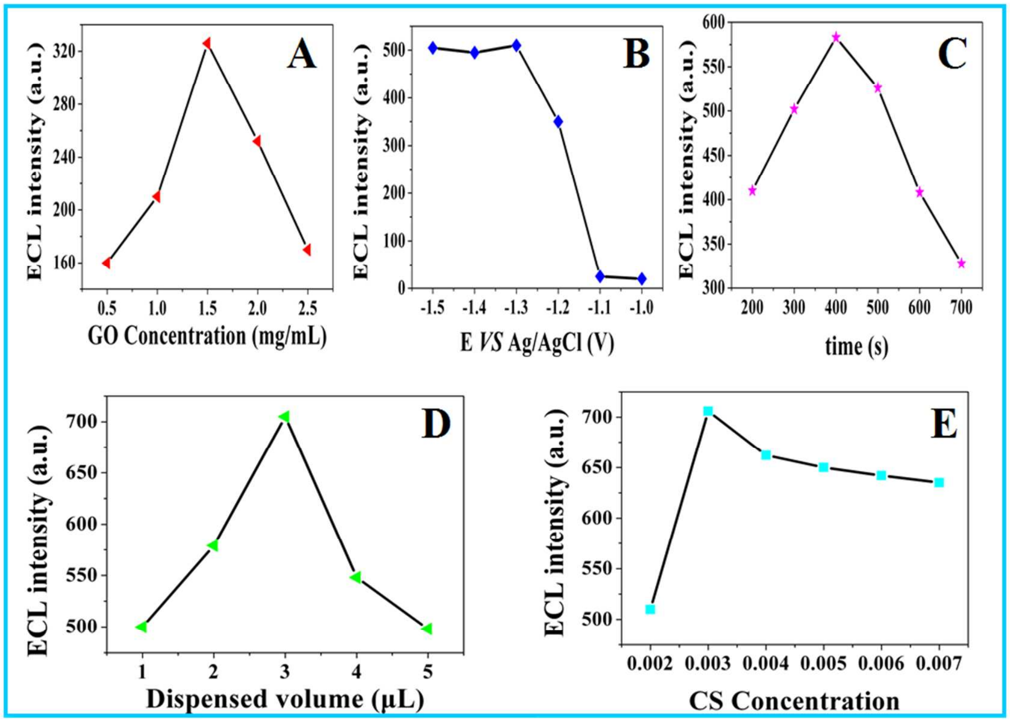

A
ECL intensity (a.u.)
160
200
240
280
320
0.5
1.0
1.5
2.0
2.5
GO Concentration (mg/mL)
B
ECL intensity (a.u.)
0
100
200
300
400
500
-1.5
-1.4
-1.3
-1.2
-1.1
-1.0
E VS Ag/AgCl (V)
C
ECL intensity (a.u.)
300
350
400
450
500
550
600
200
300
400
500
600
700
time (s)
D
ECL intensity (a.u.)
500
550
600
650
700
1
2
3
4
5
Dispensed volume (μL)
E
ECL intensity (a.u.)
500
550
600
650
700
0.002
0.003
0.004
0.005
0.006
0.007
CS Concentration

Figure 3

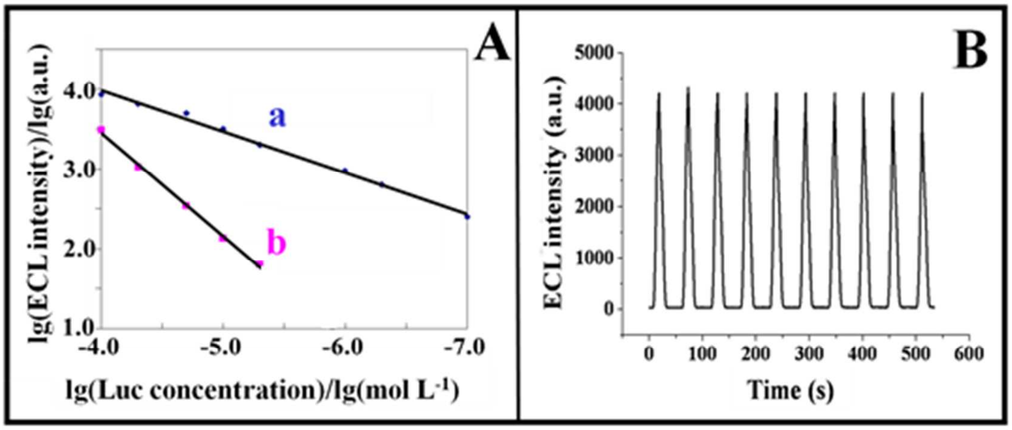
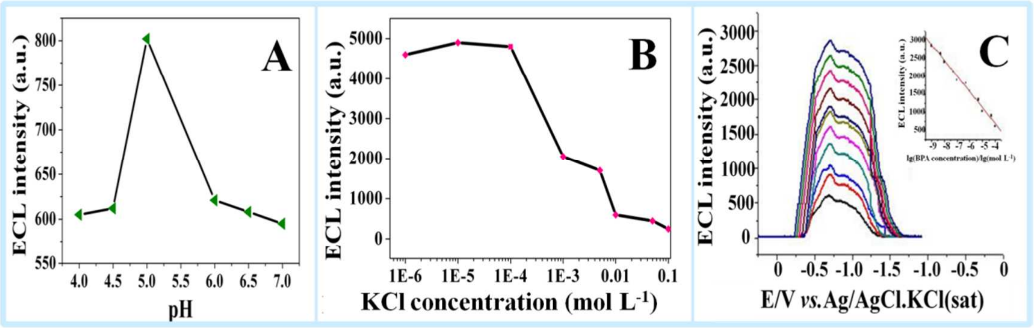

A
lg(ECL intensity)/lg(a.u.)
4.0
3.0
2.0
1.0
-4.0
-5.0
-6.0
-7.0
lg(Luc concentration)/lg(mol L-1)
a
b
B
ECL intensity (a.u.)
5000
4000
3000
2000
1000
0
0
100
200
300
400
500
600
Time (s)

Figure 4


A
ECL intensity (a.u.)
800
750
700
650
600
550
4.0 4.5 5.0 5.5 6.0 6.5 7.0
pH
B
ECL intensity (a.u.)
5000
4000
3000
2000
1000
0
1E-6 1E-5 1E-4 1E-3 0.01 0.1
KCl concentration (mol L-1)
C
ECL intensity (a.u.)
3000
2500
2000
1500
1000
500
0
0 -0.5 -1.0 -1.5 -1.0 -0.5 0
E/V vs. Ag/AgCl.KCl(sat)
lg(BPA concentration)/lg(mol L-1)

An amplified and stable electrochemiluminescent signal of lucigenin on electrochemically reduced graphene oxide contained sensing platform was obtained and exhibited obvious analytical properties towards bisphenol A.