Highly Sensitive and Simultaneous Determination of Hydroquinone and Catechol at Thionine/Graphene Oxide Modified Glassy Carbon Electrodes

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A highly sensitive electrochemical method for the simultaneous and quantitative detection of hydroquinone (HQ) and catechol (CC) was developed, based on covalent immobilization of thionine (TH) by Schiff-base reaction between –NH2 of TH and –COH onto graphene oxide modified glassy carbon electrodes (GO/GCE). The modified electrode showed excellent electrocatalytic activity and reversibility toward the oxidation of both HQ and CC in 0.1 M phosphate buffer solution (PBS, pH 7.0). The redox responses from the mixture of HQ and CC were clearly resolved in both cyclic voltammetry (CV) and differential pulse voltammetry (DPV) with the peak differences of 100 mV and 110 mV, respectively. The oxidation peak currents of HQ and CC were linear over the range from 1 to 200 μM in the presence of 150 μM of counterpart. The detection limits (S/N = 3) for HQ and CC were 20 and 25 nM, respectively. Furthermore, the electrochemical performances with some interference were studied. The developed sensor was successfully examined for real sample analysis with tap water and revealed stable and reliable recovery data.

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Hydroquinone (HQ) and catechol (CC) are two dihydroxybenzene isomers and can be used in many fields, such as tanning, cosmetics, dye, chemical, and pharmaceutical industries. At the same time, they are considered as environmental pollutants by the US Environmental Protection Agency (EPA) and the European Union (EU) due to their high toxicity and low degradability in the ecological system. They have similar basic quinine structures and coexist in environmental samples. Therefore, it is significant to establish simple and rapid analytical methods to detect them sensitively, selectively and simultaneously.

At present, the main analytical methods to determine HQ and CC are high performance liquid chromatography, spectrophotometry, electrochemiluminescence, synchronous fluorescence and electrochemical methods. However, the chromatographic method is complicated and generally takes quite a long time for a single analysis because of extra steps for pretreatment and separation before the signal detection. Optical methods usually require an additional reagent for the signal generation. Moreover, the actual effect of resorcinol on the chemiluminescence reaction is still unknown and affects the detection of HQ and CC. Therefore, electrochemical techniques, especially voltammetric methods, due to having more simplicity and selectivity are preferable over chromatographic and optical methods and more feasible for miniaturization of analysis.

Till now, high degree of overlapping between their voltammograms is one of the main barricades for most conventional solid electrodes. Also, the use of unmodified electrodes for the detection of these compounds has a number of limitations such as slow electron transfer kinetics, low sensitivity and reproducibility over a wide range of solution compositions. Hence, it is pertinent to explore and develop a reliable method to fabricate modified electrodes with electron transfer mediators. Carbon nanotube (CNT) is one of the most attractive materials to modify electrodes for simultaneous determination of HQ and CC. Qi and Zhang reported that the GCE modified by multiwall CNTs (MWCNTs), functionalized by carboxylic acid, can separate oxidation peaks of HQ and CC. Consequently, they used multi-electrode array modified with MWCNTs and lowered the detection limits. The CNT/poly(3-methyllthiophene) composite film modified electrodes were reported, while the background current was relatively high. Mesporous carbon modified electrode was reported with high sensitivity, however, it may suffer from mechanical instability because the electrode was constructed by adsorption of carbon material on substrates.

Since the discovery of graphene, which is a basic building block for graphitic materials of all other dimensionalities such as CNTs and fullerences, it is considered as a promising nanostructured carbon material by professor Andre Geim in 2004 and the first application of graphene based advanced materials has been utilized in various fields and the related references have expanded dramatically. Right now, graphene oxide (GO) can be easily synthesized using Hummer’s method, which has opened new avenue for biosensor. Moreover, because of containing a range of reactive oxygen functional groups, it could be dispersed readily in water, breaking up into macroscopic flakes, subsequently to modify electrode surface. For instance, Lu and coworkers reported a versatile method based on unprotected GO for DNA detection. Sheng et al. developed PVP-coated GO for ochratoxin A determination. Other groups have also tested the GO platform to detect other analytes, such as, Ag+, ATP, etc.

Thionine (TH), one of the family of thiazine dyes, is particularly attractive as an electroactive and photomolecular, has been extensively studied in both electrochemical biosensors and photochemical biosensors. However, the most TH-modified electrodes are unstable and irreproducible for continuous use either in substrate solution or immobilized on electrode surface. A glassy carbon electrode (GCE), one of the stable and robust carbon-based electrodes, has been widely used as substrate for polymer modification because of the higher biocompatibility with less tendency of surface fouling and a relatively low level of residual current over a wide potential range. Lately, Xingyong Xu proposed a novel protocol for effectively covalent immobilization of thionine based on Schiff-base reaction between –NH2 of TH and –COH which was in situ generated on GCE via potentiostatic activation. Surely activated GCE had good catalytic activity to oxidation of HQ and CC because the generated oxygen-containing groups are responsible for the increase of the electrochemical signals, while reversely, preanodization damaged GCE seriously and reduced its lifetime.

Here, we covalently immobilized TH onto GO modified GCE as a rapid and simple sensor for simultaneous detection of HQ and CC without any pretreatment or separation steps. Differential pulse voltammetry is used for detection of proposed analytes at micromolar concentration range. This electrode exhibited attractive performances for simultaneous determination of HQ and CC, such as sensitive and fast voltammetric response, as well as good selectivity and catalytic activity.
Figure 1. CVs of HQ(A) and CC(B) (0.5 mM of each); CVs(C) and DPVs(D) of a mixture of HQ and CC (150 μM of each) in pH 7.0 PBS at different electrodes. Scan rate: 100 mVs⁻¹.

Experimental

All reagents were of analytical grade and were used without further purification. Thionine, hydroquinone, catechol, Na₂HPO₄ and NaH₂PO₄ were purchased from Sigma–Aldrich (USA). Natural graphite powders were purchased from Alfa Aesar. Phosphate buffer solution (PBS, 0.1 M) was prepared by mixing 0.1 M Na₂HPO₄ and NaH₂PO₄. All solutions were prepared with deionized water (DI, 18 MΩcm⁻¹) obtained from a Xuefeng water purifying system (P.R. China).

Electrochemical experiments were conducted on a CHI660A electrochemical workstation (Shanghai CH Instruments Company, China). Electrochemical measurements were performed in a three-electrode cell system with a bare GCE (3 mm diameter) or modified GCE, a platinum wire, and a Ag/AgCl (3 M KCl) electrode were used as working, counter and reference electrodes, respectively. All potentials in this study referred to this reference electrode. Differential pulse voltammograms (DPV) were obtained by scanning the potential from 0 to 0.4 V with the following pulse amplitude; 100 mV/s, pulse width; 2 ms, and pulse period; 1000 ms. All electrochemical experiments were performed in 0.1 M PBS (pH = 7.0) at room temperature, unless otherwise noted.

GO was synthesized from graphite powder according to the modified Hummers method. Then, the as-synthesized graphite oxide was subjected to ultrasonication for 1 h (200 W). Finally, a homogeneous GO dispersion was obtained. Prior to modification, the GCE was carefully polished with 0.05 μm alumina on polishing cloth, and then ultrasonicated in water bath for 10 min to remove adsorbed particles. GO modified GCE (GO/GCE) was prepared by casting 3 μL GO dispersion onto the pretreated GCE surface and allowed to dry at room temperature. Following Xu’s procedure, for covalent immobilization of TH, the obtained electrode above was immersed in 1.0 M TH solution (PBS pH 7.0) for 1 h, and then rinsed with DI water to remove non-specifically adsorbed TH. The as-prepared electrode was denoted as TH-GO/GCE and stored at 4°C when not in use.

Results and Discussion

The electrochemical behavior of HQ and CC at a bare GCE, GO/GCE and TH-GO/GCE in 0.1 M PBS (pH = 7.0) were studied. Fig. 1A and 1B show the cyclic voltammograms (CVs) obtained for 0.5 mM of HQ and CC at different electrodes. At TH-GO/GCE, the redox peaks were measured at 0.14/0.06 and 0.23/0.17 V, respectively, for HQ and CC. It indicates that the redox reactions of both were reversible at the TH-GO/GCE, while obviously quasi-reversible at the bare GCE.

The electrochemical behavior of a mixture of HQ and CC (150 μM of each) was also investigated. As can be seen in Fig. 1C, at the bare GCE, there was only a broad oxidation peak at +0.28 V in CV, indicating the oxidation peaks of HQ and CC overlapped and it was impossible to separate these two compounds easily at the bare GCE. In contrast, at the TH-GO/GCE, the oxidation peaks of HQ and CC were negatively shifted and clearly resolved, located at 0.11 and 0.21 V, respectively with the peak separation of 0.1 V. In the case of DPV (Fig. 1D), the peak potentials are 0.08 and 0.19 V with the peak separation of 0.11 V. The peak separation is large enough for detection of both HQ and CC simultaneously. Similar phenomena were observed at GO/GCE whereas with comparatively low peak currents. Overall, we noted that the peak potential shifted negatively at the TH-GO/GCE and the current signals for these two components were completely resolved due to a difference in the degree of potential shift for HQ and CC. The enhanced resolution for the simultaneous detection of HQ and CC can be attributed partly to the degree of electrostatic interaction between HQ/CC and NH₂ on electrode surface through hydrogen bonding.
The effect of solution pH on the response of HQ and CC at TH-GO/GCE was investigated by CV in PBS containing HQ(A) or CC(B) (0.5 mM of each) over a pH range of 3.0–9.0 as shown in Fig. 2. We observed that the peak potential shifted negatively with the increase of solution pH for both HQ and CC. The linear regression equations (inset of Fig. 2) were $E_p$(mV) = 600.73–61.13pH ($r^2 = 0.99$), for HQ, and $E_p$(mV) = 698.56–61.17pH ($r^2 = 0.99$) for CC. The slopes of the regression equations which are close to $\sim$59 mVpH$^{-1}$ suggest that the electrode reactions of HQ and CC are both two-proton coupled two-electron transfer process.36 Meanwhile, from 3.0 to 7.0, the peak current increased gradually and over 7.0 it decreased. This could be attributed to the proton taking part in the electrochemical reaction. At pH over 7.0, HQ and CC become deprotonated as anions and they start to be oxidized.11 The electrochemical reaction becomes more difficult due to the shortage of proton. Also, HQ and CC are easily turned into anions giving rise to the electrostatic repulsion between HQ/CC and the electrode surface, which could make the peak current decrease. In order to achieve high sensitivity and considering the physiological value, pH 7.0 was chosen for the simultaneous detection of HQ and CC.

The effect of scan rate on the oxidation peak current of HQ and CC at TH-GO/GCE were shown in Fig. 3. The oxidation peak potentials of solution pH for both HQ and CC. The linear regression equations (inset of Fig. 2) were $E_p$(mV) = 407.241.231.82

![Figure 2](image_url) 

**Figure 2.** CVs of 0.5 mM HQ(A) or CC(B) at TH-GO/GCE in PBS with different pH values (right to left: 3.5, 4.4, 6.4, 7.0, 8.0, 8.35). Insets show the Plots of peak potentials vs. pH for HQ and CC, respectively.

![Figure 3](image_url) 

**Figure 3.** CVs of HQ(A) and CC(B) (0.5 mM of each) in pH 7.0 PBS at TH-GO/GCE with different scan rates (a–i: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 250, 300, and 400 mVs$^{-1}$). Insets show the plots of peak current vs. square root of scan rates for HQ and CC, respectively.

To investigate the applicability of the proposed method for the simultaneous determination of HQ and CC, local tap water samples...
were used for quantitative analysis. The spike and recovery experiments were performed by measuring DPV responses for tap water samples in which known concentrations of HQ and CC were added. Their amounts were then determined by calibration and are summarized in Table II. The recoveries for HQ and CC were 98.0–101.0% and 96.0–100.6%, respectively, which clearly indicate the applicability and reliability of the proposed method.

The interference experiments were done at TH-GO/GCE (Fig. 5). Some organic compounds such as resorcinol, phenol and nitrophenol would be interference for the simultaneous determination of HQ and CC. We investigated 100 fold resorcinol, nitrophenol, 3-nitrophenol, 4-nitrophenol and phenol, all these peak appeared between +0.5 V and +0.8 V while the peak potential of HQ and CC is at +0.05 ∼ +0.2 V which indicated that there was no interference with the quantitative detection of HQ and CC.

| Samples | Counterpart containing | Spiked(µM) | Found(µM)* | Recovery (%) |
|---------|------------------------|------------|------------|--------------|
| HQ 1    | 5.0 µM CC              | 5.0        | 4.9        | 98.0         |
| 2       |                        | 10.0       | 10.1       | 101.0        |
| 3       |                        | 15.0       | 14.7       | 98.0         |
| CC 1    | 5.0 µM HQ              | 5.0        | 4.8        | 96.0         |
| 2       |                        | 10.0       | 9.8        | 98.0         |
| 3       |                        | 15.0       | 15.1       | 100.6        |

*Average of five measurements

Figure 5. CVs of phenol, resorcinol, 2-nitrophenol, 3-nitrophenol and 4-nitrophenol (0.5 mM of each) in pH 7.0 PBS at TH-GO/GCE.

Table I. Comparison of different carbon based electrochemical sensors for the determination of HQ and CC (technique DPV).

| Electrode materials                     | Linear range (µM) | Detection limit (µM) |
|----------------------------------------|-------------------|----------------------|
| HQ                                     | CC                |                      |
| Graphene–chitosan/GCE                  | 1–400             | 0.75                 |
| Polydopamine-RGO/GCE                   | 1–2500            | 0.062                |
| Graphitic mesoporous carbon/GCE        | 2–50              | 0.37                 |
| GO–mesoporous MnO₂/GCE                 | 0.01–0.7          | 0.007                |
| Graphene–P4VP/GCE                      | 0.1–10            | 0.0081               |
| Electrochemically RGO/pentacene–CNT/GCE| 0.01–200          | 0.00761              |
| Graphene/GCE                           | 1–50              | 0.015                |
| SWCNT/GCE                              | 0.4–10            | 0.12                 |
| MWNTs/GCE                              | 1–100             | 0.75                 |
| Three-D graphene/MWCNTs/BMIMP6/GCE     | 0.5–2900          | 0.1                  |
| Graphene-GO/GCE                        | 0.5–300           | 0.16                 |
| Graphene/BMIMP6/GCE                    | 0.01              | 0.02                 |
| PEDOT/GO/GCE                           | 2.5–200           | 1.6                  |
| Thermally reduced GO/GCE               | 0.8–100           | 0.75                 |
| Pr/ZrO2-reduced GO/GCE                 | 1–1000            | 0.4                  |
| TH-GO/GCE                              | 1–200             | 0.02                 |

Table II. Recovery results for HQ and CC in tap water.

![Graph](image)

Figure 4. DPVs of HQ(A) and CC(B) with different concentrations (1, 3, 5, 10, 20, 40, 80, 100, 150, 200 µM) in the presence of 150 µM counterpart. Insets show the calibration plots of HQ and CC, respectively.
Conclusions

Electroactive TH molecules were covalently immobilized onto graphene oxide modified GCE surface by Schiff-base reaction between –NH of TH and –COOH of graphene oxide. Owing to its unique structural features and excellent electrochemical properties, the resulting TH-Go/GCE offers efficient electron transfer, wide linear range, low detection limit, high sensitivity, good reproducibility toward the oxidation of HQ and CC and potential application in real samples analysis. Valid response to HQ and CC obtained in present work also promise to the prospective performances of this method to other analytes.

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