Sensitive and Simultaneous Electrochemical Sensing for Three Dihydroxybenzene Isomers Based on Poly(L-arginine) Modified Glassy Carbon Electrode

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The simultaneous and sensitive electrochemical detection of dihydroxybenzene isomers (hydroquinone, HQ; catechol, CC; resorcinol, RS) is of great significance because such isomers can be awfully harmful to the environment and human health. In this paper, by preparing poly(L-arginine) modified glassy carbon electrode (P-L-Arg/GCE) with a simple method, a highly sensitive electrochemical sensor for simultaneously detecting HQ, CC and RS was constructed successfully due to the large surface area, good electronic properties and catalytic ability of P-L-Arg/GCE and the electrostatic action between P-L-Arg (positive) and targets (negative). Under the optimized conditions, the results show that the P-L-Arg/GCE has a wide linear range from 0.1 to 110.0 μM for HQ, CC and RS. The detection limits for HQ, CC and RS are 0.01, 0.03 and 0.1 μM, respectively. Finally, the proposed sensor was successfully applied in real sample analysis.

Keywords Dihydroxybenzene isomers, hydroquinone, catechol, resorcinol, L-arginine, electrochemical sensor, modified electrode

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GCE in addition to the electrostatic action between P-L-Arg (positive) and targets (negative), the results show that the obtained P-L-Arg/GCE effectively separated the voltammetric signals of three isomers with enhanced peak currents compared to bare GCE.

Experimental

Chemicals and instruments
HQ, CC and RS were obtained from Aladdin Reagent Co., Ltd (Shanghai, China). All the other reagents were of analytic grade without further purification. All other chemicals were of analytical grade and used directly without further purification. The supporting electrolyte was 0.1 M phosphate buffer solution (PBS) prepared with Na2HPO4 and NaH2PO4. Aqueous solutions used throughout were prepared with ultra pure water (>18 MΩ cm) obtained from a Millipore system.

All the electrochemical experiments were performed with a CHI 660E electrochemical workstation (Shanghai Chenhua Co., China). The electrochemical experiments use a three-electrode system consisting of a Ag/AgCl electrode (saturated KCl aqueous solution) as the reference electrode, a platinum wire as the counter electrode and the GCE with a diameter of 3 mm served as the working electrode. All potentials were calculated respect to the Ag/AgCl electrode. The assay unit was stirred with a magnetic stirrer and electrochemical experiments were tested at room temperature.

Fabrication of the modified electrode
The experimental conditions of the electro-polymerization were similar to those described in the paper reported previously. Briefly, the bare GCE was polished with 0.3 and 0.05 μm alumina slurry, respectively, and then cleaned ultrasonically in doubly distilled water and ethanol, respectively. After that, the GCE was immersed into the 0.1 M PBS (pH 6.0) containing 2.5 mM L-Arg. Then, the cyclic voltammetry (CV) was performed in the potential range between –2.0 and 2.5 V at a scan rate of 100 mVs⁻¹ for a predetermined number of cycles. Finally, the resulting P-L-Arg/GCE was washed with ultra pure water carefully for further use.

The simultaneous determination of HQ, CC and RS was carried out in PBS, and the determination consisted of two consecutive steps: (1) preconcentration of target molecules from the solution to the modified electrodes with stirring at a definite accumulation time, and (2) electrochemical measurements by CV and differential pulse voltammetry (DPV).

Results and Discussion

Characterization of modified electrodes
Figure 1 illustrates the surface morphology of bare GCE and P-L-Arg/GCE investigated by scanning electron microscope (SEM). A smooth surface was clearly observed on the bare GCE, whereas a granule-shaped film was observed after electropolymerization of L-Arg, which confirmed the GCE was coated by P-L-Arg film, leading to the change in the surface activity of the P-L-Arg/GCE.

The electrochemical performances of different electrodes were characterized by CV in a solution containing 5.0 mM [Fe(CN)6]3–/4– and 0.1 M KCl. was used to test the bare GCE and P-L-Arg/GCE under the CVs. As shown in Fig. 2, both GCE and P-L-Arg/GCE showed reversible and symmetric voltammograms response. Compared with the bare GCE, the response current of P-L-Arg/GCE increases 1.6-fold. These results indicate that the P-L-Arg film on GCE surface could accelerate the transfer rate of electron.

Electrochemical response of HQ, CC and RS
In order to investigate the electrochemical sensing performance of the produced P-L-Arg/GCE, the bare GCE and P-L-Arg/GCE were investigated by CV in PBS with and without three dihydroxybenzene isomers (Fig. 3). For the bare GCE, no oxidation peak could be observed in the absence of analytes (curve a); when three analytes (10.0 μM HQ, 10.0 μM CC and
10.0 μM RS) were added into the PBS, two weak oxidation peaks of HQ at ~0.145 V and CC at ~0.237 V appeared (curve b), and partially overlap with each other owing to the similar electrochemical behavior of HQ and CC, and meanwhile, two reduction peaks were observed at ~0.064 and ~0.166 V, respectively, corresponding to the reduction of the oxidation products of HQ and CC, respectively. However, for the L-Arg/GCE measured in PBS containing 10.0 μM HQ, 10.0 μM CC and 10.0 μM RS (curve c), the oxidation peaks of HQ and CC appear at ~0.126 and ~0.231 V, respectively, the peak separation is up to 105 mV, and the peak currents show much higher than those at the bare GCE. Furthermore, the oxidation peak of RS was found at ~0.625 V. These suggest that the P-L-Arg/GCE has excellent electrochemical sensing property and could be used for the simultaneous determination of HQ, CC and RS.

The schematic illustration of preparing the P-L-Arg/GCE and

![Scheme 1 Schematic illustration of electrochemically sensing dihydroxybenzene isomers based on P-L-Arg/GCE.](image)

Fig. 3  CV responses of bare GCE in PBS without (a) and with three analytes (10.0 μM HQ, 10.0 μM CC and 10.0 μM RS) (b); CV responses of the P-L-Arg/GCE in PBS containing 10.0 μM HQ, 10.0 μM CC and 10.0 μM RS (c).

![Figure 3](image)

Fig. 4  CV response of 10.0 μM HQ, CC and RS with different scan rate in the range of 20 – 200 mV/s (A); oxidation peak current of HQ (B), CC (C) and RS (D) vs. scan rates.
Mechanistically, the relationship between target and an electrode can be probed by considering the voltammetric response of functional electrode at different scan rates. Figure 4A shows the influence of scan rate on the CV response of the P-L-Arg/GCE sensor to 10.0 μM HQ, CC and RS. As indicated in the figure, both the anodic and cathodic peak current of isomers increased gradually with the increasing scan rate. Whereas, the anodic peak potentials shifted slowly to more positive and cathodic peak potential to more negative. Figures 4B – 4D shows that the response of the anodic peak current of HQ, CC and RS increased linearly with scan rate. The resulting regression equation is $I_{\text{HQ}}(\mu \text{A}) = 0.0098v(\text{mV/s}) + 0.0605$ ($R^2 = 0.9981$), $I_{\text{CC}}(\mu \text{A}) = 0.0086v(\text{mV/s}) + 0.0873$ ($R^2 = 0.999$), and $I_{\text{RS}}(\mu \text{A}) = 0.0047v(\text{mV/s}) + 0.0003$ ($R^2 = 0.995$). The linearity between current response and scan rate signifies that the electrochemical reaction of HQ, CC and RS on the P-L-Arg/GCE sensor is controlled by surface adsorption.

Optimization of experimental parameters

As a highly sensitive electrochemical technology, DPV has much higher current sensitivity and better resolution compared to CV. So the optimization of the conditions was investigated by DPV to obtain high sensitivity for the determination of analytes, and the corresponding results are shown in Fig. 5. The curves a, b and c are for RS, CC and HQ, respectively. We studied the effect of the electrolyte pH on the electrochemical response of HQ, CC and RS at P-L-Arg/GCE. Figure 5A shows the obtained current response of different pH in the range of 5.0 – 9.0. With the increase of pH values, the current response of HQ, CC and RS increased linearly up to the highest peak at the pH value of 6.0 and decreased when the value exceeded 6.0. Moreover, the oxidation peak potentials of HQ, CC and RS shifted negatively when increasing the value of pH, revealing that proton takes part in their oxidation. The linear regression equations for HQ, CC and RS were $E_{\text{pa}}(\text{V}) = 0.4170 – 0.055\text{pH}$ ($R^2 = 0.9833$), $E_{\text{pa}}(\text{V}) = 0.5580 – 0.0589\text{pH}$ ($R^2 = 0.9845$) and $E_{\text{pa}}(\text{V}) = 0.9460 – 0.0740\text{pH}$ ($R^2 = 0.9870$), respectively. The other factor of pH condition is the current response separation of HQ and CC. It was found from Fig. 5B...
that the curve of each target is degressive when increasing value and the $\Delta E$ of each value of pH is shows a similar trend. Based on the above, pH 6.0 was chosen as the optimized condition for the experiments.

In the electropolymerization, the number of polymerization cycles must be considered. After electropolymerization of L-Arg on the GCE surface with a certain number of cycles, the obtained P-L-Arg/GCE was used to measure the electrochemical response of HQ, CC and RS. As shown in Fig. 5C, the response of peak currents of the three analytes increased when increasing the number of cycles starting from 2, the max current was noted at 10 cycles and decreased as the number of cycles was further increased. As shown in Fig. 5D, HQ, CC and RS show similar responses. As a result, 10 cycles was finally chosen as the optimized experiment condition.

The influence of accumulation time of P-L-Arg/GCE in PBS containing HQ, CC and RS was also investigated by DPV. Figures 5E and 5F shows the relationship between current response and accumulation time. When the accumulation time increased from 25 to 300 s, it was found that the max current value was at 100 s and the response decreased as accumulation time exceeded 100 s. Also, there were no clear differences between the separation of peaks. Considering the comprehensive condition, 100 s was selected as the optimal condition during the experiment.

From Figs. 5C and 5E, it was also found that the peak current of three analytes are different, and the peak current response of RS at $-0.53$ V is much smaller than those of HQ at $-0.09$ and CC at $-0.2$ V owing to its weak electroactivity comparing to HQ and CC at P-L-Arg/GCE.

Electrochemical sensing of HQ, CC and RS on the P-L-Arg/GCE

Under the optimum conditions, the P-L-Arg/GCE was used to simultaneously detect HQ, CC and RS. Figure 6A shows the DPV responses of HQ, CC and RS with different concentrations from 0.1 to 110.0 $\mu$M. It is worthy to note that weak peak separation between HQ and CC occurred upon the increased concentration of the solution. Therefore, no further efforts were made to interrogate objective analytic concentration levels. The calibration curves were established by drawing $I$ versus $C$ of HQ, CC and RS. Figure 6 (B, C and D) shows the regression curves of HQ, CC and RS, respectively, of the equations as follows: $I(\mu A) = 0.0661C(\mu M) + 2.4049$ ($R = 0.993$), $I(\mu A) = 0.0506C(\mu M) + 2.0514$ ($R = 0.994$) and $I(\mu A) = 0.0066C(\mu M) + 0.2929$ ($R = 0.999$). Relevantly, the limits of detection (LOD) of HQ, CC and RS were 0.01, 0.03 and 0.1 $\mu$M, respectively. The analytic results are listed in Table 1. From the table, it was found that the sensing performance obtained by the proposed method is much better than most reported in previous papers. Importantly, the proposed method for the simultaneous detection of HQ, CC and RS is very simple, low cost, rapid and easy to operate.

The reproducibility and stability of the P-L-Arg/GCE were evaluated by detecting the current response on 10.0 $\mu$M HQ, CC and RS. The results showed that the modified electrode possesses a satisfying reproducibility with a relative standard deviation (RSD) of less than 6.5% for 10 parallel experiments with different electrodes. Also, after the modified electrode was stored at room temperature for 30 days, the current decrease was only about 4.78%. The above results demonstrate that the P-L-Arg/GCE is suitable for the analysis of HQ, CC and RS in...
The results suggest that no interference was observed for methanol, ethanol, fenpropathrin, o-chlorophenol, and d-phenylalanine, suggesting that the developed electrode is suitable for the routine analysis of three dihydroxybenzene isomers in practical samples. The interference substances during the detection of HQ, CC and RS at P-L-Arg/GCE were also investigated by the addition of various ions and pollutants to PBS in the presence of 2.0 μM HQ, CC and RS. The results suggest that no interference was observed for the following inorganic ions: Na⁺, K⁺, Mg²⁺, Ca²⁺, Zn²⁺, Al³⁺, Cl⁻, SO₄²⁻ and NO₃⁻ (50 equiv.); similarly, there was no interference observed for methanol, ethanol, fenpropathrin, pentachlorophenol, L-tyrosine, o-chlorophenol, and d-phenylalanine, suggesting that the developed electrode is suitable for the routine analysis of three dihydroxybenzene isomers in practical samples.

Analytical application in real samples

In order to test the modified electrode for the detection of HQ, CC and RS at P-L-Arg/GCE, revealing that the improved method is feasible for the determination of HQ and CC and RS in real samples.

Conclusions

In summary, by producing a P-L-Arg/GCE and using it for the first time to detect HQ, CC and RS simultaneously, sensitive electrochemical sensor for three dihydroxybenzene isomers was constructed successfully. Under optimized conditions, the linear range for the three targets was 0.1 to 110 μM, and the LOD of HQ, CC and RS were 0.01, 0.03 and 0.1 μM, respectively. Also, the proposed electrochemical sensor exhibits good reproducibility, stability and fast response. Furthermore, compared to the previous reports for the electrochemical determination of dihydroxybenzene isomers, the proposed method in this paper is very simple, low cost, rapid and easy to operate. It is believed that the P-L-Arg/GCE holds promise for applications in electrochemical sensing for HQ, CC and RS analysis.

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