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A Simple and Sensitive Electrochemical Sensor Based on Azodicarbonamide for the Determination of Tert-Butylhydroquinone in Food

Xinying Ma, Meifeng Chen, Shouqing Xue[*]*, Huiping Lv, Qi Zhu, Fenghai Li*

College of Chemistry and ChemicalEngineering of HezeUniversity, 2269 N. DaxueRoad, Heze,Shandong, 274015,P.R.China.
[*]E-mail: hezexueyuanhx@163.com

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An electrochemical sensor for the determination of the antioxidant tert-butyl hydroquinone (TBHQ) in food was prepared by using a modified electrode based onazodicarbonamide (ADC) on a glassy carbon electrode (GCE) surface. The electrochemical behavior of TBHQ on the modified electrode was studied by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results showed that the peak redox current was significantly stronger than that on the bare GCE and that the detection sensitivity was improved. The experimental conditions, including the electrochemical polymerization pH value of the ADC solution, scanning rate, voltage, sweep segments and pH value of the TBHQ solution were optimized. Under optimal conditions, TBHQ presented a linear relationship with the oxidation peak current within the concentration range of 4.00×10^{-7}~4.00×10^{-4} mol/L; the correlation coefficient was R=0.9988, and the limit of detection was 1.80×10^{-9} mol/L. The modified electrode was applied for the determination of real food samples containing TBHQ, with recovery ranging between 96.5 and 103.0%. This electrochemical method showed good selectivity and high sensitivity.

Keywords: azodicarbonamide; glassy carbon electrode; tert-butyl hydroquinone

1. INTRODUCTION

Tert-butylhydroquinone (TBHQ) is a common synthetic phenolic antioxidant that is often added to food products to prevent oxidative degradation of oils and fats. Typical products in which TBHQ isused are cookies, edible oil, fried flour products, cooked nuts, raisins, fruit pastries, margarine, prepared food, etc. [1]. TBHQ also has shown good results in the preservation of biodiesel samples [2-4]. Although TBHQ can be well used in preserving food product quality, an excess is considered a health risk, and its usage is strictly confined in most countries [5]. The Food and Drug Administration (FDA) and European Union (EU) have evaluated TBHQ and permitted safe usage levels. However, at

higher doses, it is harmful to lab animals because it can produce precursors to stomach tumors and even damage DNA [6]. Therefore, it is necessary to establish a simple, accurate and sensitive method for the detection of TBHQ. Some methods have been used for analysis of TBHQ, including gas chromatography (GC) [7, 8], fourier-transform infrared (FTIR) spectroscopy [9], high-performance liquid chromatography (HPLC) [10,11], flow-injection analysis (FIA) [12], photoelectrochemical (PEC) [13] and electrochemical methods [14-25]. HPLC and GC have some disadvantages, such as a complicated process, high cost, long detection times, and the fact that organic solvents can also pollute the environment. The electrochemical analysis method has the advantages of sensitivity, good selectivity and low cost; some literature has reported the use of such a method by using electrodes modified by two or more modifiers; however, the preparation was complex, and the reproducibility and stability of the method was poor.

Azodicarbonamide (ADC) is commonly used as a blowing agent in the industry and as a flour treatment agent. However, it has not been reported in the literature for the electrochemical modification of a GCE. In this study, an ADC/GCE which has high conductivity for the detection of TBHQ, was fabricated by an electrochemical method. The electrochemical behavior of TBHQ on the modified electrode was studied and a new method was established for the determination of TBHQ in food. The modified electrode was prepared with only one modifier, and stable performance and good reproducibility were found; the method showed some advantages, including high sensitivity, simple operation, a wide linear range and good selectivity, and was used for the determination of TBHQ in fried chips, biscuits and roasted nuts with satisfactory results.

2. MATERIALS AND METHODS

2.1 Instruments and reagents

The following instrumentation and related reagents were used: an electrochemical workstation (CHI660D, Shanghai Chenhua Instrument Co., Ltd); three electrode system, with a saturated Ag/AgCl electrode as the reference electrode, a platinum wire electrode as the pair electrode, and GCE or ADC/GCE as the working electrode; KQ-100 ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd); quartz subboiling high pure water distiller (s SYZ-550, Jintan Jiangnan instrument factory); microinjector; circulating water three-tap multipurpose vacuum pump (SHZ-CB, Henan Yuzhang Industry Co., Ltd); a field emission scanning electron microscope (JEOL-JSM-6700F, Japanese Electronics Company); quantitative filter paper (Φ of 12.5 cm, Hangzhou Special Paper Industry Co., Ltd).

ADC was purchased from Sigma-Aldrich; TBHQ was purchased from Aladdin. $K_3[Fe(CN)]_6$ was obtained from Aladdin. KCl was obtained from Chengdu Aikeda Chemical Reagent Co., Ltd. The pH of phosphate buffer solution (PBS) was varied by using Na_2HPO_4 (0.2 mol/L) and citric acid (0.1 mol/L). Fried chips, biscuits and roasted nut samples were purchased from a local supermarket. All the reagents used were analytically pure. Distilled water was obtained from quartz subboiling and used in experiments.

2.2 Process of sample treatment

A 50.0 g sample of fried chips was crushed. The sample was dissolved with 50 mL anhydrous ethanol. After ultrasonic treatment for 5 minutes, a vacuum pump was used to extract the filter residue. Then, 20 mL of anhydrous ethanol was added to the filter residue. The twice filtrate was transferred into a 100 mL volumetric flask to prepare the sample solution.

The sample processing method used for biscuits and roasted nuts was the same as that used for the fried chips.

2.3 Preparation of ADC/GCE

The GCE was burnished on metallographic paper and polished on suede with an alumina suspension, which was followed by ultrasonic cleaning in nitric acid (1:1), anhydrous ethanol and distilled water for 30 s. Then, the electrode was scanned in a 5.0 mmol /L $K_3[Fe(CN)]_6$ solution containing 0.1 mol/L KCl by CV to clean and activate the electrode until the peak current was basically stable and the current difference was less than 80 mV. The CV method was used to scan the electrodes for 16 sweep segments in pH 7.0 PBS containing 1.0 mmol /L ADC within the potential range of -1.6 ~ 2.4 V at a scan rate of 100 mV/s.

2.4 The experimental method

A certain volume of TBHQ standard solution (pH 2.2 PBS) and buffer solution were placed into the electrolytic cell. A three-electrode system was used to study the electrochemical behavior of TBHQ, and optimize the experimental conditions by CV within the potential range of -0.5 ~ 1.0 V at a scan rate of 100 mV/s or by DPV within the potential range of 0.1 ~ 0.8 V on the ADC/GCE.

3. RESULTS AND DISCUSSION

3.1 Optimum electrochemical polymerization conditions

The preparation conditions for the modified electrode affect the performance and sensitivity of the modified electrode. The influence of the pH, sweep segments, range of polymerization potential and scan rate on the determination of TBHQ by ADC/GCE was discussed. The results showed that the current determined by ADC/GCE for TBHQ was the highest for a pH of 7.0, number of sweep segments of 16, polymerization potential range of -1.6~2.4 V, and scan rate of 100 mV/s. These parameters are similar to the factors found to influence the preparation conditions of a poly(aminosulfonic acid)-modified glassy carbon electrode and poly(L-tryptophan)-modified glassy carbon electrode [26, 27].

3.2 Cyclic voltammograms (CVs) for the polymerization of ADC/GCE

Figure 1 shows the results obtained from the CVs of ADC/GCE under optimal polymerization conditions. It can be seen from the figure that a pair of redox peaks appear in the first cycle of polymerization; as the number of scan cycles increased, the peak current increased, but the increase in the amplitude decreased. Finally, the redox peaks were stable, indicating that electrochemical reaction of ADC occurred on the electrode surface.

Figure 2 shows the scanning electron microscope images of the GCE (A) and ADC /GCE (B). It can be seen from the figure that a uniform and dense modified film was formed on the surface of the GCE; the surface structure of ADC /GCE is unlike that found for an electrode modified by a polymer film [26-28].

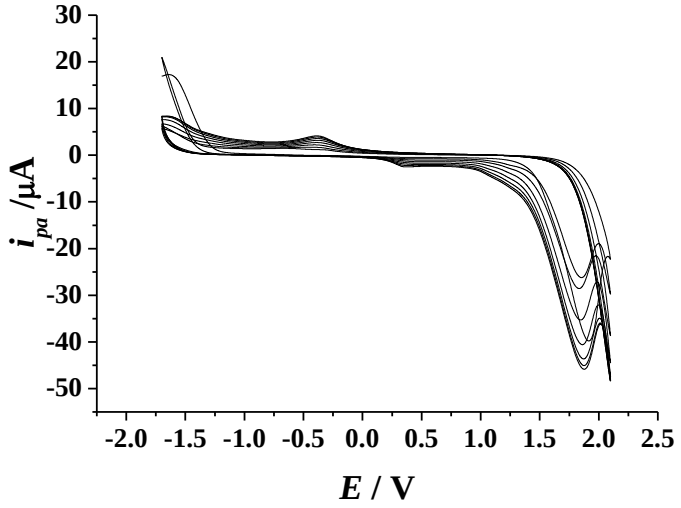


Figure 1. CVs of1.00×10^{-4}mol/L ADC polymerization

The pH was 7.0, the number of sweep segment was 16, the range of polymerization potential was -1.6~2.4 V, and the scan rate was 100 mV/s

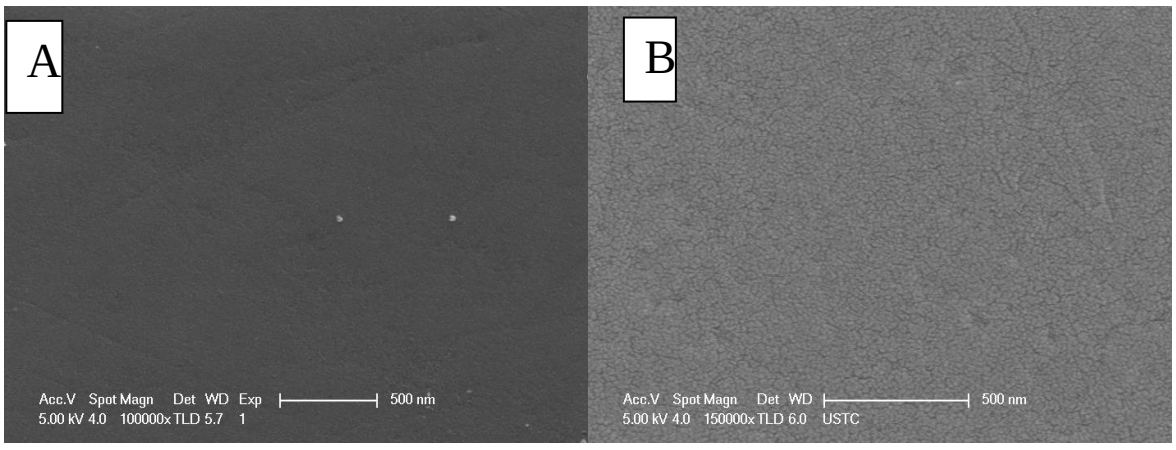


Figure 2. SEM images of GCE (A) and ADC /GCE (B)

3.3 Electrochemical behavior of TBHQ

Figure 3 shows the CVs obtained for TBHQ at GCE (a), ADC/GCE (b) and ADC/GCE in PBS (5.0) (c). It can be seen from Figure 3a that the oxidation peak current (i_{pa}=-0.87 μA) and reduction peak current (i_{pc}=0.78 μA) for TBHQ at the GCE are smaller than that at the ADC/GCE (i_{pa}=-3.72 μA， i_{pc}=2.10 μA); the oxidation peak current at ADC/GCE is 4.2 times higher than that of the bare electrode. The results showed that the modified membrane for ADC had a certain catalytic effect on the electrochemical reaction of TBHQ, which accelerated the electron transfer rate. The peak potentials were observed to be E_{pa}=0.48 V and E_{pc}=0.09 V, respectively, The reaction of TBHQ at the ADC/GCE (b) is a quasi-reversible reaction because i_{pa}/i_{pc}=1.77and ΔE=0.39 V. According to the Nernstian equation and Laviron theory[29, 30], a possible redox process for TBHQ at the ADC/GCE can be inferred that is similar to the reaction process of TBHQ on glassy carbon electrodes modified with gold nanoparticles [6, 18], as shown in scheme 1.

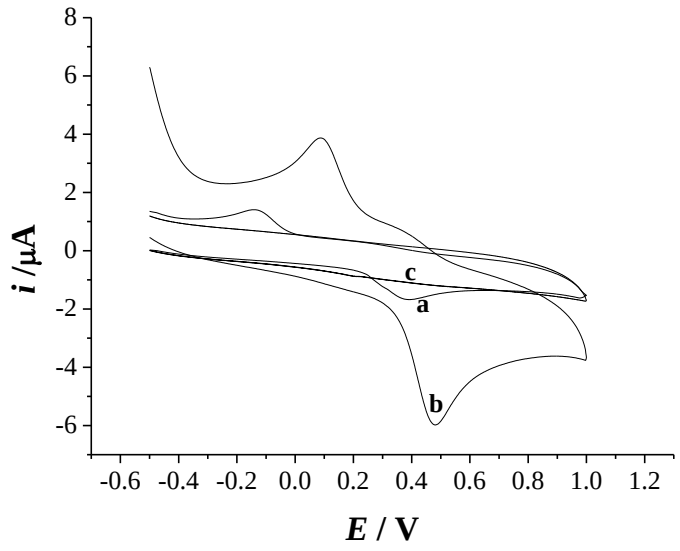
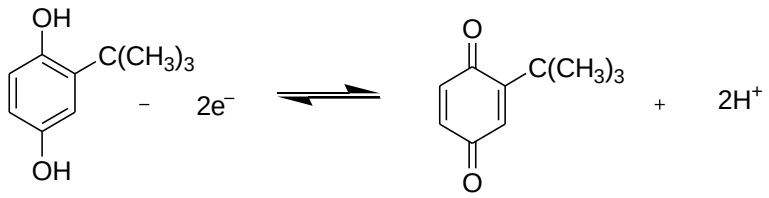


Figure 3. CVs of1.00×10^{-4}mol/L TBHQ at bare GCE (a),ADC/GCE (b) and ADC/GCE in PBS (pH=2.2) (c), scan rate is 100 mV/s



Scheme 1. The oxidation reaction equation of TBHQ at the modified electrode

3.4 Optimal experimental conditions for the determination of TBHQ

3.4.1 Effect of pH

The oxidation behaviors of TBHQ in different PBS solutions with pH varying from 2.2 to 8.0 were studied to discuss the influence of the pH value. As seen in Figure 4, the oxidation peak currents for TBHQ decreased gradually with pH value from 2.2 to 8.0; so, pH 2.2 PBS was selected in this study. Experiments also showed a negative shift in oxidation peak potential for increasing pH from 2.2 to 8.0. The relationship between the oxidation peak potentials and pH was found to be described by a linear regression equation. The linear regression equation was:E_{pa}=0.68-0.057pH with a correlation coefficient ofR=0.9985. The slope was determined to be 0.057 V, which is close to a value of 0.059 V and could be attributed to the fact that protons are involved in the process of TBHQ oxidation. Moreover, according to the Nernstian equation: E∝59.16 m/n pH (m represents the proton-transfer number and n represents the electron transfer number), the number of protons transferred was equal to the number of electrons transferred [24,29].

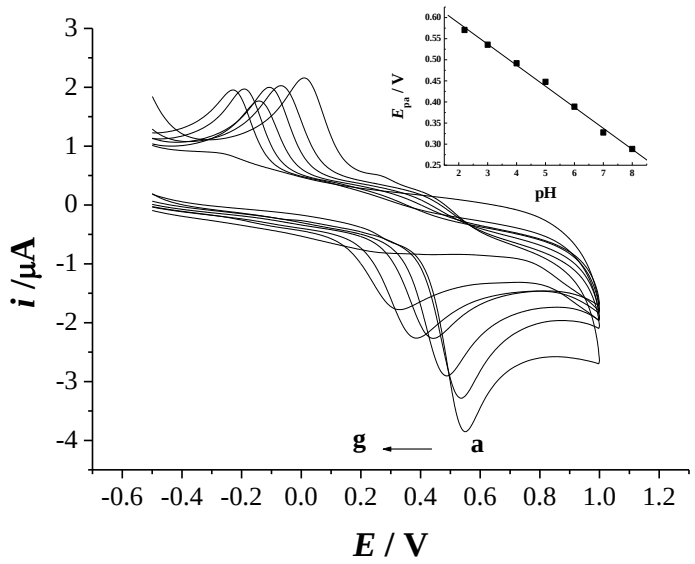


Figure 4. CVs of 1.0×10^{-4}mol/LTBHQ in PBS of different pH values (a tog: 2.2, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) on the ADC/GCE. Insert is the plot of peak potential of TBHQ vs. pH value of PBS. Scan rate is 100 mV/s.

3.4.2 Effect of scan rate

Within the potential range of -0.6 V to 1.0 V, the scan rate was changed for the experiment. The results showed that the oxidation behavior of TBHQ changed with varying scan rate. It can be seen from Figure 5 that the oxidation peak increased with increasing scan rate. The peak shape was good when the scan rate was 100 mV/s. A scan rate of 100 mV/s was selected in this experiment; moreover, if the scan rate was too low or large, the detection limit of the sample was reduced, which affected the

result of the experiment. The oxidation and reduction peak currents and the square root of the scan rate show linearity (illustration shown in Figure 5) that can be described with linear regression equations expressed as i_{pa}=-7.66×10^{-6}+9.23×10$^{-8}v^{1/2}$, R=0.9998, i_{pc}=4.21×10^{-6}-1.04×10$^{-7}v^{1/2}$, R=0.9928, respectively. These results indicate that the electrode process for TBHQ on the ADC/GCE was a diffusion process[24]; this result is similar to the electrochemical behavior of TBHQ found for a Au–SnO_2/graphene-single-walled carbon nanotube nanocomposite-modified glass carbon electrode [19].

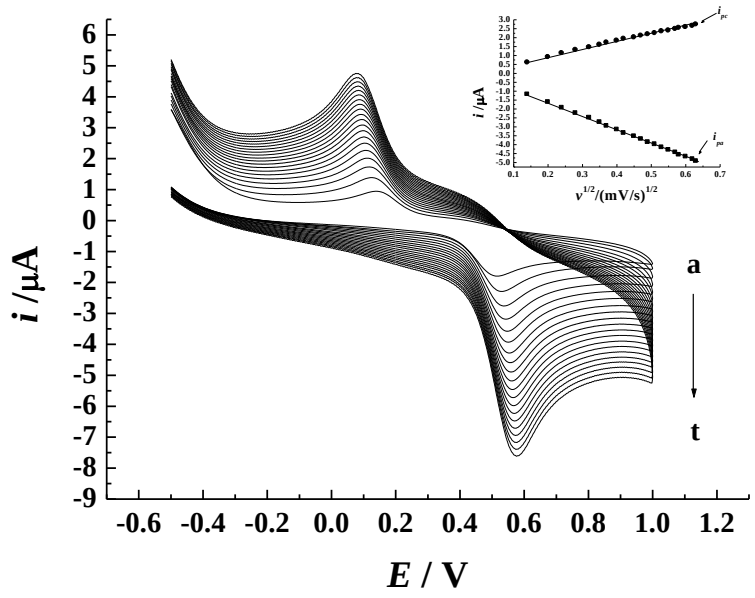


Figure 5. CVs of 1.00×10^{-4}mol/L TBHQ in PBS(pH2.2) of different scan rate at ADC/GCE . Oxidation and reduction peak currents and the square root of scan rate show the linearity (Insert). Scan rate from a to t corresponds to 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400 mV/s.

Figure 6 shows plots of the peak potential versus the logarithm of the scan rate. The linear regression equations for E_{pa} and E_{pc} versus the scan rate are expressed as:

E_{pa}=0.59+0.047log$v$(mV/s), R=0.9972 (1);E_{pc}=0.063-0.045log$v$(mV/s), R=-0.9958 (2)

According to Laviron theory [30], $E_{pa} = a + (2.303RT/(1-\alpha)n_{\alpha}F)\log v$ (mV/s)(1)

$E_{pc} = b - (2.303RT/\alpha n_{\alpha}F)\log v$ (mV/s)(2)

where*a* and *b* are constants, *F and R*are the Faraday constant (96,487 C) and universal gas constant (8.314 J·K $^{-1}$·mol $^{-1}$), respectively; *T* is the Kelvin temperature (K) and *n* is the number of electrons transferred and α is the electron transfer coefficient. By combining Eqs. (1) and (2), we obtained n_{α}=2.57, and α=0.51. According to the literature [6, 26] and the linear regression equation for the oxidation peak potential (E_{pa}) for varying pH: E_{pa}=0.68-0.057pH, the electron transfer number for the oxidation reaction of TBHQ should be 2, and the proton transfer number should also be 2. Here, α is quite similar to its theoretical value of 0.5. Therefore, the electrode process for TBHQ on the ADC/GCE was a quasi-reversible process.

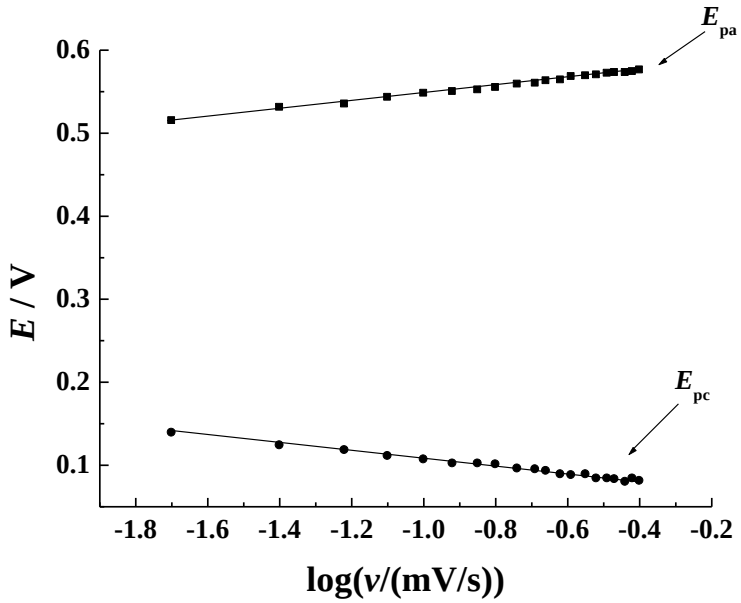


Figure 6. Linearity of the peak potentials of 1.00×10^{-4} mol/L TBHQ versus the logarithms of the scan rates

3.5 Working curve linearity range, limit of detection

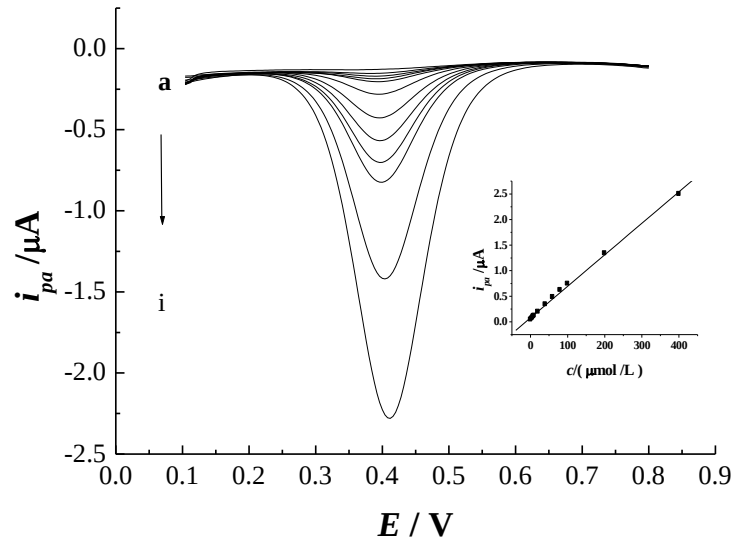


Figure 7. DPVs of different concentrations of TBHQ at ADC/GCEin PBS(pH2.2). Letters from a toi correspond to concentration of 0.4, 6.0, 10, 20, 40, 60, 80, 100 μmol/L. Insert is the plot of the oxidation peak current versus concentration of TBHQ.

Under optimized conditions,the working curve linearity range was investigated by CV, DPV and linear sweep voltammetry (LSV), respectively. The results showed that the DPV method was better than the other methods, which showed the widest range of high sensitivity. Therefore, the DPV

method was selected in this work. From Figure 7, we can see that the oxidation peak current for TBHQ increases linearly with TBHQ concentration in the range of 4.00×10^{-7}~4.00×10^{-4} mol/L, and the linear function can be expressed asi_{pa}=7.90×10^{-8}+0.0062c (mol/L), R=0.9988. The limit of detection was determined to be 1.8×10^{-9} mol/L. Table 1 shows a comparison between the linear range and detection limit of TBHQ determined by this method and the reported electrochemical method. As seen from the table, the detection limit for the current method is relatively low, and the linear working range is relatively wide compared with the methods reported in the references.

Table1. Comparison of different electrodes for TBHQ

Electrode	Method	Linear range (μmol•L^{-1})	LOD (μmol•L^{-1})	Reference
SPE–MWCNT	LSV	0.5-10	0.34	14
CPE	LSV	1.05-10.15	0.0711	15
HMDE	SWV	1.05-10.15	0.0343	16
AuNPs/GCE	LSV	1-16.8	0.47	18
Au–SnO2 /GN-SWCNT/GCE	DPV	0.05-230	0.058	19
Pt/PPy/NiPcTs	DPV	20-140	0.74	21
MIP-PdAuNPs-ERGO/GCE	DPV	3-360	0.28	24
CPE/BMIB/ZnO-CNTs	LSV	0.09–750	0.05	25
ADC/GCE	DPV	0.4-400	0.0018	This work

3.6 Reproducibility and stability of electrodes

Under optimal experimental conditions, 4.00×10^{-5}mol/L TBHQ was measured six times by the DPV method, and the relative standard deviation RSD=1.8% (n=6) was obtained. The current for the oxidation peak of 8.00×10^{-5}mol/L TBHQ was maintained at 98.5%, 97.4% and 97.1% compared with the DPVs obtained previously at selected time intervals (5 days, 10 days, and 20 days) by using the same modified electrode, indicating that the modified electrode was stable.

3.7 Interference experiment

Under optimal experimental conditions,the influence of some other matter on the determination of 4.0×10^{-5} mol/L TBHQ was investigated. The results show that relative errors were allowed within the range of 5% for 100 times sucrose, KCl and NaCl, 50 times Cu^{2+} and Ca^{2+}, 20 times citric yellow

and sunset yellow, and 50 times butylated hydroxyanisole (BHA) and dibutylhydroxy toluene (BHT), which did not interfere with the determination.

3.8 Sample determination

The recovery rate of the sample was determined under the best experimental conditions by DPV. The experimental results are shown in Table 2.

Table 2. Recovery measurements of the TBHQ in food samples (n=6)

Sample	Added ($\times10^{-6}$ mol/L)	Total ($\times10^{-6}$ mol/L)	Recovery(%)	RSD(%)
fried chips	-	8.12	-	2.6
	4.00	11.98	96.5	1.9
	8.00	16.01	98.6	2.5
	12.00	20.19	100.5	1.7
biscuits	-	9.56	-	2.1
	4.00	13.68	103.0	2.7
	8.00	17.68	101.5	1.5
	12.00	21.84	102.3	2.6
roasted nuts	-	4.54	-	1.9
	2.00	6.48	97.0	2.1
	4.00	8.59	101.3	1.7
	8.00	12.35	97.6	2.7

PBS (pH=2.2)

The content of TBHQ was calculated to be 0.0045 g/kg, 0.0064 g/kg, and 0.0032 g/L in fried chips, biscuits, and roasted nuts, respectively.

4. CONCLUSIONS

An ADC/GCE prepared using a simple method was shown to have a significant catalytic effect on the electrochemical oxidation of TBHQ. Compared with a bare electrode, the oxidation peak current was significantly increased, and the sensitivity for the determination method was improved. The oxidation peak current for TBHQ presented a good linear relationship with concentration within the range of 4.00×10^{-7}~4.00×10^{-4}mol/L, and the detection limit was determined to be 1.80×10^{-9}mol/L. The modified electrode was applied for the detection of TBHQ in actual food samples, and good results were obtained, with recovery ranging between 96.5 ~103.0%.

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