

Simple and Rapid Determination of Tartrazine Using Poly(L-arginine)/Electrochemically Reduced Graphene Oxide Modified Glassy Carbon Electrode

Peilong Wang^{1,2}, Xu Liu¹, Qingqing Hu¹, Hui Gao^{1,*} and Wei Ma^{1,*}

¹ School of Chemistry and Materials Science, Huaibei Normal University, Huaibei, Anhui 235000, China

² Information School, Huaibei Normal University, Huaibei, Anhui 235000, China

*E-mail: gaohuichem@chnu.edu.cn

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A simple and rapid electrochemical method based on a poly(L-arginine)-electrochemically reduced graphene oxide-modified glassy carbon electrode fabricated by cyclic voltammetry has been developed for the determination of tartrazine. Compared with the bare glassy carbon electrode, the modified electrode achieved a well-defined and enhanced oxidation peak due to the increased conductivity and electrochemical active surface area. The bare glassy carbon electrode and the modified electrode were characterized by scanning electron microscopy and electrochemical impedance spectroscopy. The electrocatalytic oxidation behaviors of tartrazine on the modified electrode were investigated using cyclic voltammetry and differential pulse voltammetry. The voltammetric peak current of tartrazine exhibits good linearity in the range of 1.00×10^{-6} – 2.50×10^{-4} mol L⁻¹ under the optimal differential pulse voltammetry conditions, with a detection limit of 2.5×10^{-7} mol L⁻¹ (at an S/N of 3). This proposed method has also been applied for quantitative analysis of tartrazine in some carbonated beverage and fruit juice samples, with satisfactory results.

Keywords: L-arginine, reduced graphene, tartrazine, modified electrode Synopsis (graphical abstract):

1. INTRODUCTION

Tartrazine, a synthetic organic food colourant, is added to processed foodstuffs including soft drinks, fruit juice, dairy products, bakery products, and so on to improve its visual aesthetics, stability, and anti-pollution properties [1]. However, the toxicity of tartrazine has been determined by some published data. According to the study of Sasaki et al. [2], 2000 mg kg⁻¹ tartrazine in the mouse colon 24 h can damage DNA. Studies also indicate that tartrazine has been related to human health problems especially in neurobehavioral parameters [3], allergies, thyroid tumours, diarrhea, vomiting, urticaria,

and overactive behaviour in children [4]. This colorant has been evaluated by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) [5]. In China, the maximum amount of tartrazine permitted in food stuffs is 0.1 g kg^{-1} (GB2760-1996) [6]. Consequently, analysis of tartrazine in food stuffs by accurate and reliable methods is generally necessary.

To date, various methods such as high-performance liquid chromatography–mass spectrometry (HPLC–MS) [7-10], spectrophotometry [11-15], and capillary electrophoresis [16] have been proposed for the quantification of tartrazine. In addition, an electrochemical method that possesses the advantages of high sensitivity [17], economical instrumentation, and convenient and rapid detection procedures is an alternative way to determine tartrazine [18]. For this method, the properties of the electrode modifiers are a vital factor. The reported electrode modifiers used for the determination of tartrazine include reduced graphene oxide with Au nanoparticles [19], β -cyclodextrin-coated poly(diallyldimethylammoniumchloride)-functionalized graphene [20], poly(5-sulfosalicylic acid)/Cu(OH)₂ nanoparticles [21], multi-walled carbon nanotubes [22], Pt nanoparticles [23], graphene oxide and multi-walled carbon nanotubes nanocomposite [24], and polyallylamine [25]. Among these modifiers, graphene functionalized by doping with suitable materials such as Au nanoparticles and multi-walled carbon nanotubes exhibits good electrochemical performance. In recent years, amino acids, which have both carboxylic acid and amine functional groups, have also been used to functionalize graphene based on their easy availability and polymerization onto the electrode surface [26-28]. Nevertheless, to the best of our knowledge, electrodes modified with reduced graphene oxide and amino acid composite films has never been applied for the measurement of tartrazine.

In this study, a poly(L-arginine)-electrochemically reduced graphene oxide-modified glassy carbon electrode (PLA-ERGO/GCE) was established to detect tartrazine. The electrochemically reduced graphene oxide and L-arginine composite film was deposited on the electrode surface by the electropolymerization method and characterized by scanning electron microscopy. Cyclic voltammetry (CV) shows that the PLA-ERGO/GCE exhibited remarkable electrochemical activity for tartrazine oxidation, which was validated according to the increased anodic peak current. Moreover, tartrazine was quantified by differential pulse voltammetry (DPV), showing good sensitivity. Furthermore, this detection method has promising application in some carbonated beverage and fruit juice samples.

2. EXPERIMENTAL

2.1 Reagents and Materials

Tartrazine, L-arginine and graphene oxide (2 mg mL^{-1}) was purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China), Bio Life Science & Technology Co. (Shanghai, China), and XFNANO Materials Technology Co. (Nanjing, China), respectively. The $1.00 \times 10^{-3} \text{ mol L}^{-1}$ tartrazine stock dispersion and $5.00 \times 10^{-3} \text{ mol L}^{-1}$ L-arginine stock dispersion were prepared by direct dissolution of the original samples in doubly distilled deionized water. The 1 mg mL^{-1} graphene oxide stock dispersion was prepared by a phosphate buffer solutions (PBS) at pH 5.5 (prepared by $0.1 \text{ mol L}^{-1} \text{ H}_3\text{PO}_4$, NaOH, and $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$). All of the above stock dispersion solutions were stored in dark. The tartrazine

standard solutions (1.00×10^{-6} – 2.50×10^{-4} mol L⁻¹) were diluted with PBS immediately before use. All chemical reagents used in this work were of analytical grade. All aqueous solutions were prepared by doubly distilled deionized water. All measurements were performed at room temperature (23.0 ± 2.0 °C).

2.2 Apparatus

All electrochemical measurements throughout this study were performed on a BAS100B/W electrochemical workstation (BAS group, USA), except that electrochemical impedance spectroscopy (EIS) was performed on a Zahner Zennium electrochemical workstation (Germany). The conventional three-electrode system included a glassy carbon electrode (3 mm diameter) serving as the working electrode, a platinum wire electrode serving as the auxiliary electrode, and a KCl-saturated Ag/AgCl electrode serving as the reference electrode. The scanning electron microscopy (SEM) used in this work was a Quanta 450 microscope (JSM-6610LV, JEOL Company, Japan). The acidity of all the solutions' was measured with a digital pH/mV meter (PHS-3C, Shanghai Kang Yi Instrument Co., Ltd, China).

2.3 Fabrication of PLA-ERGO/GCE

After mechanically polishing with 0.05 μm Al₂O₃ powder, the bare glassy carbon electrode (GCE) with mirror-like smoothness was washed thoroughly with nitric acid (1:1) and ethanol. For further cleaned, the electrode was then immersed in doubly distilled deionized water to sonicate 10 min. Finally, it was placed in air to dry naturally. Removal of physically adsorbed materials from the electrode surface, the clean GCE was transferred to 10 mL polymerization solution containing 5 mL 1 mg mL⁻¹ graphene oxide stock dispersion, 2.50 mL 5×10^{-3} mol L⁻¹ L-arginine stock dispersion, and 2.50 mL pH 5.5 PBS. Then, electro polymerization process was conducted by cyclic sweeping from a potential range from 2.5 V to -2.4 V at 120 mV s⁻¹ for 8 cycles after 5 s quiet time. Following electrode position, the modified electrode was rinsed with doubly distilled deionized water carefully. After dry in air, the PLA-ERGO/GCE modified electrode was ready for subsequent electrochemical studies.

2.4 Electrochemical Measurements

To obtain a stable voltammogram before electrolysis, the PLA-ERGO/GCE modified electrode was immersed in 10 mL blank solution (5 mL pH 7.0 PBS and 5 mL doubly distilled deionized water) to activate using cyclic voltammetry by scanning the potential from -0.2 V to 0.8 V for 1 cycle at a scan rate of 180 mV s⁻¹. Cyclic voltammetric measurements were subsequently performed in 10 mL buffer solution (5 mL pH 2.0 PBS and 5 mL tartrazine standard solutions at the proper concentrations). The scan was carried out between 0.4 V and 1.4 V at a scan rate of 100 mV s⁻¹, and the quiet time was 240 s. To avoid contamination of the electrode with adsorptive substances, the PLA-ERGO/GCE modified electrode was cleaned with doubly distilled deionized water after every measurement. Before every measurement, the PLA-ERGO/GCE was activated again in the abovementioned blank solution with the same CV procedure. The optimized parameters used in DPV were a 8 mV potential increment, 60 ms

pulse width, 40 mV pulse amplitude, and 100 ms pulse interval.

3. RESULTS AND DISCUSSION

3.1 Characterization of PLA-ERGO/GCE

To improve the sensitivity of this method, the electroanalytical signals were investigated at different polymerization potential ranges with a bare GCE in pH 5.5 PBS solution containing the graphene oxide stock dispersion and L-arginine stock dispersion. When the negative potential was relatively small, only a pair of reversible redox peaks of L-arginine appeared, with an oxidation potential of 1.7 V and reduction potential -0.6 V (which was in accord with that reported [29]). Moreover, the peak currents gradually decreased when the values of the negative potential decreased. As the negative potential value increased above 2.1 V, a new oxidation peak of graphene appeared at 0.5 V indicating that the graphene was reduced, and the corresponding peak currents increased, representing an increase in sensitivity. In the forward scan, the reduced graphene was oxidized, resulting in an oxidation peak at 0.5 V. The highest sensitivity and good stability of the modified electrode were obtained after scanning 8 times in the potential range from 2.5 V to -2.4 V at a scan rate of 120 mV s $^{-1}$.

Scanning electron microscopy images of the GCE, PLA/GCE, and PLA-ERGO/GCE are shown in Figure 1. Compared with the morphology of the bare GCE, that of the PLA film exhibits a smooth and uniform surface, with even coverage on the electrode surface. Slight crumpling and wrinkling on the PLA-ERGO/GCE surface are clearly observed, which is attributed to the π - π interaction of ERGO [30]. Since this wrinkled nature provides more electron access to the electrode surface, the electron transfer ability of the PLA-ERGO/GCE was enhanced [31].

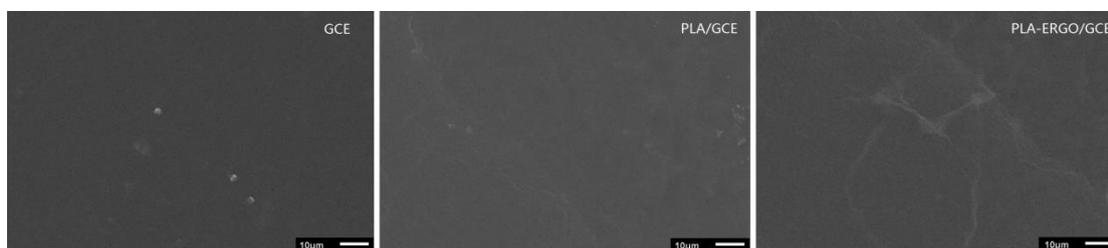


Figure 1. SEM images of (a) GCE, (b) PLA/GCE and (c) PLA-ERGO/GCE.

EIS analysis, using $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe, which is sensitive to the carbon-based electrode surface [32, 33], was applied to evaluate the electron transfer kinetics of the bare GCE and PLA-ERGO/GCE. Figure 2 depicts the corresponding Nyquist plots obtained at the bare GCE (curve a) and the PLA-ERGO/GCE (curve b). For bare GCE, a large defined semicircle represents a higher R_{ct} value and resistance. Moreover, the semicircle at high frequencies was found to be significantly smaller for the PLA-ERGO/GCE, indicating excellent conductivity. This superior electrochemical property of

PLA-ERGO/GCE was due to the larger electrode surface provided by graphene and enhancement with the L-arginine [27]. L-arginine may smooth the “wrinkles” of graphene and prevent further aggregations, which may increase the electron transfer at the interface and help to offer larger specific surface area [34].

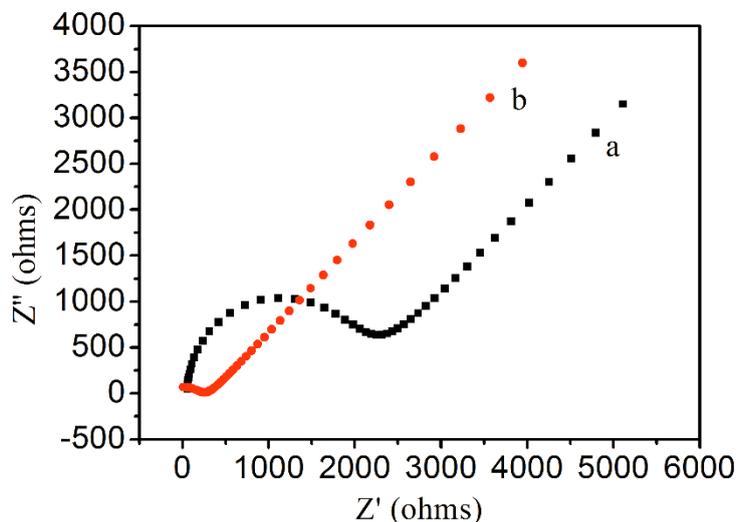


Figure 2. EIS graph of GCE (1) and PLA-ERGO/GCE(2) in $5.0 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$, $1.0 \text{ mol} \cdot \text{L}^{-1} \text{ KCl}$ solution.

3.2 Electrochemical Detection of Tartrazine Using Different Electrodes

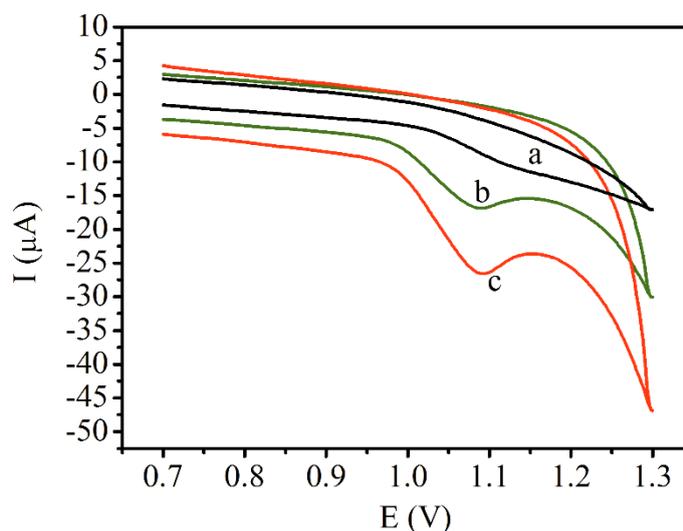


Figure 3. CVs of TT on GCE (a), PLA/GCE (b) and PLA-ERGO/GCE (c) at a scan rate of 0.10 V s^{-1} in PBS (pH 2.0).

Figure 3 compares the electrochemical activity for $1.00 \times 10^{-4} \text{ mol L}^{-1}$ tartrazine recorded at the bare GCE (curve a), PLA/GCE (curve b) and PLA-ERGO/GCE (curve c). From the cyclic voltammograms, the response of the oxidation peak was poor at the bare GCE but notably appeared at

1.0 V at the PLA/GCE and PLA-ERGO/GCE. At the PLA-ERGO/GCE, the oxidation current was relatively larger. This observation indicated an effective catalytic activity of the PLA-ERGO/GCE for tartrazine oxidation. Such efficient electrochemical activity of the PLA-ERGO/GCE is attributed to the rapid charge transfer rate co-enhanced by the graphene and amino acids. In addition, no tartrazine reduction peaks were observed for all electrodes, and this behaviour confirmed that the oxidation process of tartrazine is irreversible.

3.3 Optimum of Analytical Condition

To obtain higher sensitivity, some electrochemical voltammetric parameters such as peak potential, solution pH, scan rate were investigated to establish the optimal analytical conditions for tartrazine oxidation at the PLA/ERGO-GCE.

3.4 Effect of Scanning Potential

To determine the optimum scanning potential range, a step potential from 0.9 V to 1.7 V was first applied while maintaining the low potential at 0.5 V. The maximum oxidation current appeared at a potential of 1.3 V in the recorded CV curves. Similarly, a step potential from 0.3 V to 0.7 V was then applied while maintaining the high potential at 1.3 V, and the maximum oxidation current was observed at 0.5 V. Accordingly, the following work was performed by setting the potential scan window between 0.5 V and 1.3 V.

3.5 Effect of pH

The aqueous solution pH is an important factor in electrochemical behaviour, mainly in terms of the impact of the peak potential and peak current [35, 36]. The influence of pH on the electrocatalytical oxidation of 1.00×10^{-4} mol L⁻¹ tartrazine was studied in the pH range of 2.0–8.5 by DPV. As seen in Figure 4, the peak potential shifted negatively with increasing pH, demonstrating that protons take part in the oxidation of tartrazine [37], and the regression equation was $E_{pa}(V) = -0.03198\text{pH} + 1.08134$, $r = 0.99901$. By applying a pH value more greater than 2.0, oxidation peak currents of tartrazine decreased. Thus, pH 2.0 was chosen as the optimal value for tartrazine determination in this work.

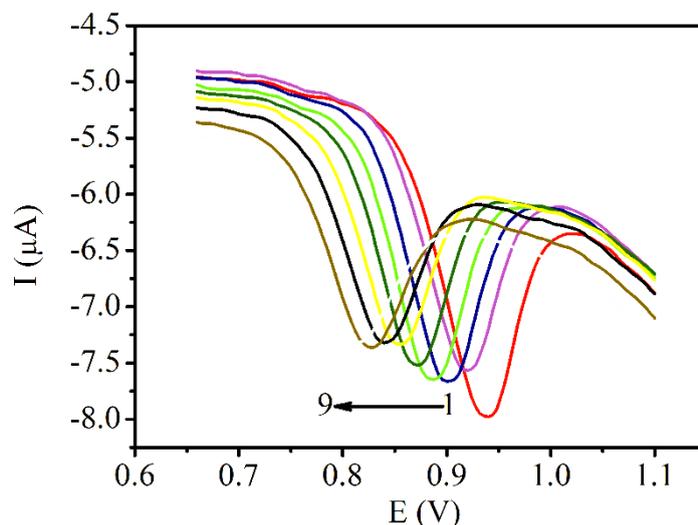


Figure 4. DPV curves of TT with different pH (from 1 to 9: 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5; scan rate of 0.10 V s^{-1}).

3.6 Effect of Scan Rate

To obtain kinetic datas of tartrazine, the relationship between peak current and scan rate was investigated. The oxidation process of $1.00 \times 10^{-4} \text{ mol L}^{-1}$ tartrazine in pH 2.0 PBS was examined by cyclic voltammetry at scan rates from 0.04 V s^{-1} to 0.40 V s^{-1} . As the scan rate increased, an increase in the peak current was observed, as shown in Figure 5. The dependence of the peak current on the scan rate can be described by the equation $\lg I_{pa} = 0.23631 + 0.5065 \lg v$, with $r = 0.9929$. It was found that the oxidation and reduction peak currents are proportional to the square root of the scan rate, indicating that diffusion control majorly contributes to the oxidation of tartaric acid on the PLA-ERGO/GCE [38]. Thus, 100 mV s^{-1} was employed as the optimum scan rate considering that the optimum oxidation peak shape could be obtained.

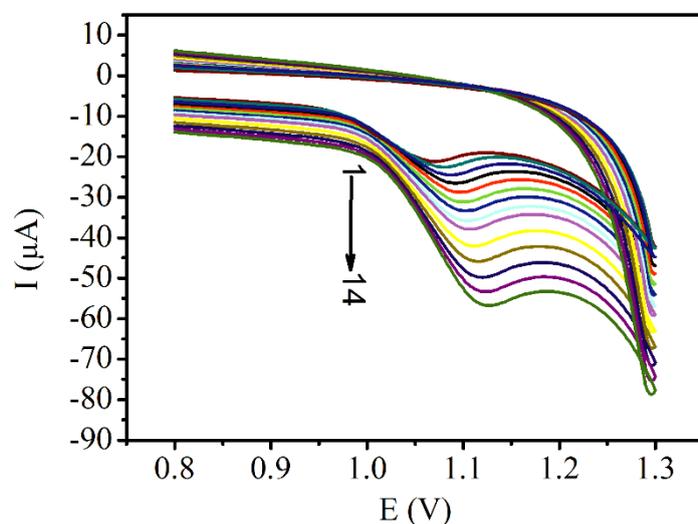


Figure 5. $1.00 \times 10^{-4} \text{ mol L}^{-1}$ TT under different CV scan rates (from 1 to 14: 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16, 0.18, 0.20, 0.24, 0.28, 0.32, 0.36, 0.40 V s^{-1}).

3.7 Quantitative Analysis of Tartrazine

Quantitative measurements of tartrazine at the PLA/ERGO-GCE were carried out using the more sensitive technique DPV, which is able to suppress back ground noise [39, 40]. The DPV plots under optimal experimental conditions are recorded in Figure 6. The peak current of tartrazine increased proportionally with concentrations from $1.00 \times 10^{-6} \text{ mol L}^{-1}$ to $2.50 \times 10^{-4} \text{ mol L}^{-1}$ with a linear function $\lg I = 1.9046 + 0.2661 \lg \rho$ ($R = 0.9909$) (Table 1), and the detection limit was $2.5 \times 10^{-7} \text{ mol L}^{-1}$ ($S/N = 3$).

Table 1. Linear ranges, regression equations, correlation coefficients and detection limits for determination of tartrazine on the PLA-ERGO/GCE

Analyte	Linear ranges $\rho / (\text{mol L}^{-1})$	Linear regression eq. $I (\mu\text{A}) \rho (\text{mol L}^{-1})$	Corr. coeff.	Detection limit (mol L^{-1})
tartrazine	$1.00 \times 10^{-6} - 2.50 \times 10^{-4}$	$\lg I = 1.9046 + 0.2661 \lg \rho$	0.9909	2.5×10^{-7}

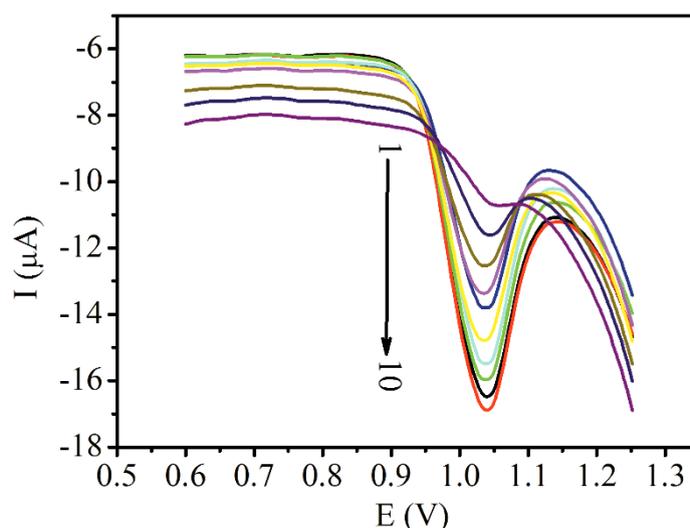


Figure 6. DPV curves of different concentrations of TT (from 1 to 10: 1.00×10^{-6} , 2.50×10^{-6} , 5.00×10^{-6} , 7.50×10^{-6} , 1.00×10^{-5} , 2.50×10^{-5} , 5.00×10^{-5} , 7.50×10^{-5} , 1.00×10^{-4} , $2.50 \times 10^{-4} \text{ mol L}^{-1}$).

The performance of the modified electrode is compared with that other sensors for tartrazine detection, and the results are shown in Table 2. It can be seen from the table that this work has more wider linear range. Compared with PL/GCE [25], the result has obvious advantages in detection limit.

Table 2. Comparison of analytical performance of present sensor with other sensors reported in the literature

Modified electrode	Linear range (μM)	Detection limit (μM)	Technique used	Reference
^a β -CD-PDDAGr	0.05-20	0.0143	DPV	[20]
^b PSSA/Cu(OH) ₂ -Gr/GCE	0.01-0.6 0.6-10	0.008	SWV	[21]
^c MWNT/GCE	0.376-75.2	0.188	DPV	[22]
^d MIP-MWNTs IL @PtNPs/GCE	0.03-5.0	0.008	DPV	[24]
^e PL/GCE	10-200	1.8	SWV	[25]
PLA-ERGO/GCE	1-250	0.25	DPV	This work

^a β -cyclodextrin-coated poly(diallyldimethylammonium chloride)-functionalized graphene composite film

^b Poly(5-sulfosalicylic acid) (PSSA)/Cu(OH)₂ nanoparticle-graphite (Gr) nanocomposite-modified glassy carbon electrode

^c multi-walled carbon nanotubes film modified glassy carbon electrode

^d multiwalled carbon nanotubes - ionic liquid supported Pt nanoparticles composite film coated glassy carbon electrode

^e polyallylamine modified tubular glassy carbon electrode

3.8 Precision and Stability

To characterize the repeatability of the proposed method, ten parallel measurements for 1.00×10^{-4} mol L⁻¹ tartrazine were carried out under the same experimental conditions, and the relative standard deviation (RSD) value was 3.4% showing satisfactory repeatability. In addition, the durability of the PLA-ERGO/GCE was investigated by monitoring the changes in the current and potential of 1.00×10^{-4} mol L⁻¹ tartrazine. After 15 days, the peak current and peak potential response showed no obvious change. This observation indicated that the tartrazine oxidation process on PLA-ERGO/GCE has long-term stability.

3.9 Interference Studies

The selectivity of this method was evaluated by interference experiments based on the determination of 1.00×10^{-4} mol L⁻¹ tartrazine together with several foreign species (relative error less than 5%). It was found that 0.4 mg Pb²⁺, 0.2 mg Cu²⁺, 0.1 mg ascorbic acid, and amounts of Na⁺, K⁺, Cl⁻, Zn²⁺, Ca²⁺, Cu²⁺, Al³⁺, starch, L-tyrosine, L-valine and L-cysteine above 1.0 mg had almost no influence on tartrazine oxidation (the upper limit of the concentration was not determined), suggesting good selectivity of this method. The above interference, in contrast, caused a decrease in *I*_p when were present in a concentration higher than the upper limit, Which could be due to a blocking effect to the access of colorants to the electrode surface [41].

3.11 Sample Analysis

To demonstrate the practical applicability of the above-presented method, it was applied to the detection of tartrazine in commercially available carbonated beverage and fruit juice samples. Properly diluted samples were determined by the standard curve method (1 mL sample solution was diluted with 9 mL pH 2.0 PBS to bring the tartrazine concentration into the linear range), and the results are listed in Table 3. The relative standard deviations of each sample for four measurements were calculated to be approximately 3%, indicating the excellent reproducibility of this method. Moreover, the recovery of this method was studied, and the value was between 98.2% and 99.6%, revealing high accuracy for tartrazine detection.

Table 3. Analytical results of tartrazine in samples (n = 4)

Analyte		Mean value $\rho / (\text{mol L}^{-1})$	RSD / %	Added $\rho / (\text{mol L}^{-1})$	Found $\rho / (\text{mol L}^{-1})$	Recovery / (%)
carbonated beverage	1	1.48×10^{-6}	3.1	5.00×10^{-6}	6.39×10^{-6}	98.2
	2	1.50×10^{-6}	2.9	5.00×10^{-6}	6.48×10^{-6}	99.6
fruit juice	1	2.46×10^{-6}	2.8	5.00×10^{-6}	7.42×10^{-6}	99.2
	2	2.51×10^{-6}	3.0	5.00×10^{-6}	7.45×10^{-6}	98.8

4. CONCLUSIONS

A simple and rapid electrochemical method with a PLA/ERGO-GCE for tartrazine detection has been developed. The PLA/ERGO-GCE shows remarkable electrocatalytic activity towards tartrazine oxidation by cyclic voltammetry and exhibits good performances in terms of the linear range and detection limit by differential pulse voltammetry. The results of interference experiments show that some common species do not interfere with tartrazine detection. The potential applications of this method were confirmed by the measurement of tartrazine in carbonated beverage and fruit juice samples. In summary, this electrochemical method is very promising for the determination of tartrazine.

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