Simultaneous determination of the isomers of Ponceau 4R and Amaranth using an expanded graphite paste electrode

Jing Zhang, Meiling Wang, Chao Shentu, Wenchang Wang, Zhidong Chen

Abstract
A sensitive and convenient electrochemical method was developed for the simultaneous determination of the isomers of Ponceau 4R and Amaranth by an expanded graphite paste electrode (EGPE). The EGPE was prepared by mixing EG with solid paraffin. Compared with flame graphite paste and carbon paste electrodes, the EGPE showed higher electrocatalytic activity towards the oxidation of Ponceau 4R and Amaranth. Under optimal experimental conditions, the EGPE exhibited wide linear responses to Ponceau 4R and Amaranth ranging from 6 × 10⁻⁸ to 4 × 10⁻⁶ mol/L and 8 × 10⁻⁸ to 4 × 10⁻⁶ mol/L, respectively, and the detection limits for Ponceau 4R and Amaranth were 2 and 36 nmol/L, respectively, at a signal-to-noise ratio of 3. The EGPE showed good reproducibility, stability and reusability. The proposed method was successfully applied in the detection of Ponceau 4R and Amaranth in grape juice samples.

Keywords: Ponceau 4R, Amaranth, Isomers, Electrochemical method, Simultaneous determination, Food analysis

1. Introduction
The isomers of Ponceau 4R and Amaranth, as synthetic azo-colorants, are widely added into foodstuffs and soft drinks to make them more appealing (Yang, Yin, & Shao, 2011). However, they are potentially toxic and pathogenic, particularly if consumed in large amounts (Minioti, Sakellariou, & Thomaidis, 2007; Wang et al., 2010). The usage of Ponceau 4R and Amaranth is strictly controlled by laws and regulations (Vidotti, Cancino, Oliveira, & Rollemberg, 2005; Yoshioka & Ichihashi, 2008). Based on the Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommendations, acceptable daily intakes are between 0 and 0.5 mg/kg for Amaranth, and 0 and 4 mg/kg for Ponceau 4R. Due to their similar structures and properties, it is a challenge to simultaneously determine the isomers of Ponceau 4R and Amaranth. Simultaneously monitoring of Ponceau 4R and Amaranth in food products is imperative to ensure adherence with legislation, and building more sensitive, practical and convenient analytic methods for simultaneous, on-site monitoring in foodstuffs is important to help assure food safety.

Based on their specific absorption, various spectrophotometric methods have been reported for the simultaneous determination of Ponceau 4R and Amaranth including high performance liquid chromatography (HPLC) (Feng et al., 2011; Ma, Luo, Chen, Su, & Yao, 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008), capillary electrophoresis (Ryvolová, Táborský, Vrábel, Krášenský, & Preisler, 2007), and spectrophotometry (Ni, Wang, & Kokot, 2009; Zalacain et al., 2005). Although these methods are well-proven and widely accepted, they require relatively expensive equipment, advanced technical expertise, and are costly and time-consuming. Due to high sensitivity, simplicity, and easy miniaturization, electrochemical techniques are the best candidates for the on-site detection of azo-colorants such as Sudan (Mo et al., 2010; Yin et al., 2011), Sunset Yellow (Nevado, Flores, & Llerena, 1997), Tartrazine (Nevado, Flores, Llerena, & Freseniux, 1997), Amaranth (Wang et al., 2010) and so on. To the best of our knowledge, the simultaneous determination of Ponceau 4R and Amaranth isomers, based on electrochemical techniques, has not been reported.

Expanded graphite (EG), a light and highly porous, worm-like material, has recently emerged as an intriguing material for potential applications in adsorbent (Zhang et al., 2012), biomedical materials (Shen et al., 1998), fuel cells (Celzard, Mareché, Furdin, & Puricelli, 2000), catalysts (Celzard, Schneider, & Marčché, 2002; Li, Han, Liu, Zhang, & Tao, 2007) and sensors (Calas-Blanchard, Noguer, Comtat, Mauran, & Marty, 2003) due to its unique mechanical and electronic properties. To improve the absorption and conductivity, EG has been used as electrode material for the determination of tryptophan, catechol, and simultaneous determination of 4-chlorophenol and oxalic acid (Kong, Xu, Mao, Yao, & Ding, 2012; Manea et al., 2008; Zhao, Kong, Kan, & Chen, 2009). EG could facilitate the absorption of azo-colorants and charge...
transfer and, thus, improving electrode sensitivity. In this work, we prepared an expanded graphite paste electrode (EGPE) for the simultaneous determination of Ponceau 4R and Amaranth based on square-wave stripping voltammetry (SWSV) method.

The EGPE was prepared by mixing EG with solid paraffin. It had a porous worm-like structure and a high specific surface area. Compared with flake graphite paste electrode (FGPE) and carbon paste electrode (CPE), the EGPE enhanced electrocatalytic oxidation signals from Ponceau 4R and Amaranth. The aim of this work was to develop a fast, convenient and sensitive electrochemical method for simultaneous determination of Ponceau 4R and Amaranth. Based on the high electrocatalytic response to Ponceau 4R and Amaranth, a convenient electrochemical method is proposed for the simultaneous determination of Ponceau 4R and Amaranth at the EGPE. The effects of several parameters such as amount of solid paraffin, pH of Britton–Robinson (BR) buffer solutions, and accumulation time and potential were examined.

2. Experiment

2.1. Reagents

Ponceau 4R and Amaranth were purchased from Aladdin (Shanghai, China). Methanol of HPLC grade and ammonium acetate were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Flake graphite (FG) (99%, grain size 50 mesh) was purchased from Qingdao Haida Graphite Co. Ltd., (Qingdao, China). Graphite powder with a purity >99.85% was obtained from Colloid Chemical Co. Ltd., (Shanghai, China). Three kinds of grape juice samples (sample 1: 100% pure grape juice, Cofo Wine & Spirits Chemical Co. Ltd., Shanghai, China; sample 2: 100% pure grape juice, Yantaihuaxiadadiwinery Co. Ltd., Yantai, China; sample 3: 100% pure grape juice, Changyu Pioneer Wine Co. Ltd., Yantai, China) were purchased from a local market. Other compounds were of analytical grade. All the solutions were prepared with twice distilled water. A series of 0.05 mol/L BR buffer solutions of different pH values were prepared by adjusting the mixed acid solutions containing H3PO4, glacial acetic acid and borax with 1 mol/L NaOH solution. All the reagents were used without further purification.

2.2. Apparatus

Electrochemical experiments were performed with a CHI 660D electrochemical workstation (CH Instruments Inc., Austin, USA) with a conventional three-electrode cell. An EGPE was used as working electrode, a saturated calomel electrode (SCE) and a platinum wire were used as reference electrode and auxiliary electrode, respectively. All the electrochemical measurements were carried out at 25 °C. A pHS-2C digital pH meter obtained from Shanghai Kang Ning Electro-optic Technology Co. Ltd., (Shanghai, China) with a combined glass electrode was used to adjust pH values of the BR buffer solution, which was used as the supporting electrolyte in the voltammetric experiments. HPLC determinations of Ponceau 4R and Amaranth in grape juice samples were carried out on a HPLC system containing an UV–visible detector (model EX 1600, USA) and a pump (model LC 1600, USA). The chromatographic separation of Ponceau 4R and Amaranth was performed on an arcus EP-C18 (4.6 × 150 mm, 5 μm in diameter) column (USA) at 25 °C. The mobile phase of methanol/NH4HCOOH (20:80, v/v) was delivered at flow rate of 1 mL/min. Injection volume was 20 μL with handsampling, and the detection was monitored at 240 nm according to previous reports (Feng et al., 2011; Ma et al., 2006).

2.3. Preparation of EG

EG was prepared as previously described elsewhere (Zhao et al., 2009). FG (6 g) was mixed with 72 mL concentrated H3PO4 and 24 mL concentrated HNO3. The solution was stirred for 10 min and 1.5 g KMnO4 added. After one hour at 27 °C with stirring, the graphite intercalation was washed thoroughly with twice distilled water until neutralization, filtered and then dried in a vacuum oven for 2 h at 87 °C. The resulting graphite intercalation was rapidly expanded at 927 °C for 30 s in a muffle furnace to obtain EG.

2.4. Preparation of EGPE, FGPE and CPE

The EGPE was prepared according to the following procedure. EG was crushed and mixed uniformly with solid paraffin. The mixture was heated to 65 °C and stirred constantly to ensure uniform mixing, and cooled to room temperature. The expanded graphite paste was pressed into the end cavity (4 mm in diameter) of the electrode body, and the surface was smoothed against an 80 g A4 printing paper. Electrical contact to the pastes was established by inserting a copper wire (2 mm in diameter) down the glass tube and into the back of mixture. As a control, FGPE and CPE were prepared, using the same procedure, by mixing flake graphite or graphite power with solid paraffin, respectively.

2.5. Analytical procedure

The SWSV analytical procedure is comprised of two steps: accumulation step and stripping step. Ponceau 4R and Amaranth were accumulated on the EGPE biased at a constant potential of 0.4 V for 400 s in 0.05 mol/L BR buffer solutions (pH 7) with different concentrations of Ponceau 4R and Amaranth under stirring. After 30 s, voltammograms were recorded from 0.4 to 0.8 V. Before or after every measurement, the EGPE was regenerated by cycling voltammetry (10 times) from 0.3 to 1.0 V in 0.05 mol/L BR buffer solution (pH 6). Data for condition optimization and calibration curves were average from three measurements. After three parallel measurements in the same condition, the paste was carefully removed and a new EGPE was fabricated.

2.6. Sample detection

Different grape juice samples were purchased from a local market and used directly without any pretreatment. When detecting Ponceau 4R and Amaranth using the EGPE, 0.2 mL of grape juice was added into 20 mL pH 7 BR buffer solutions and analyzed according to the analytical procedure described in Section 2.5.

3. Results and discussion

3.1. Electrochemical behaviors of Ponceau 4R and Amaranth at EGPE, CPE and FGPE

The Square wave stripping voltammograms (SWSVs) of EGPE exhibited two stable oxidation peaks at 0.534 V and 0.684 V (Fig. 1, curve a), which corresponded to the oxidations of hydroxy groups of Ponceau 4R and Amaranth to their corresponding quinones, respectively. Although these peaks also occurred at CPE (Fig. 1, curve b) and FGPE (Fig. 1, curve c), the peak currents were 2.2 and 1.7 times lower than those of EGPE, respectively, indicating the significant sensitizing effect of EG, which leads to enhanced sensitivity for the electrochemical determination for Ponceau 4R and Amaranth. The sensitizing effect of EG may benefit from the multiporous structure and large specific surface area of...
29.2 m²/g, while the specific surface areas of the flake graphite and graphite powder were 0.55 and 2.89 m²/g, respectively.

3.2. Optimization of the condition for the EGPE fabrication

The amount of solid paraffin for the fabrication of EGPE was one important parameter affecting the EGPE performance. As shown in Fig. 2, the peak currents for Ponceau 4R and Amaranth at the resulting EGPE increased with the increasing amounts of solid paraffin, from 0 wt.% to 1 wt.%, but subsequently decreased with additional paraffin. These decreases may due to the lower electrical conductivity of the electrode caused by the higher amount of solid paraffin. Therefore, a paraffin content of 1 wt.% was selected for the preparation of EGPE.

3.3. Optimization of detection variables

3.3.1. Effect of pH

Fig. 3A displays the effect of pH on the oxidation peak currents of Ponceau 4R and Amaranth at the EGPE. With the pH increasing from 3 to 7, the currents increased, but decreased above pH 7. Therefore, pH 7 of 0.05 mol/L BR buffer solutions was used as the electrolyte in the simultaneous determination for Ponceau 4R and Amaranth. In addition, the relationship between the oxidation peak potentials of Ponceau 4R and Amaranth, at the EGPE, and pH were also constructed. As shown in Fig. 3B, with increasing pH from 3 to 10, the oxidation peak potentials of Ponceau 4R and Amaranth, at the EGPE, shifted linearly to a more negative potential. The regression equations for Ponceau 4R and Amaranth were $E_{pa}(V) = -0.051$ pH + 0.884 (R = 0.999) and $E_{pa}(V) = -0.058$ pH + 1.083 (R = 0.998), respectively, indicating that protons are directly involved in the oxidation of Ponceau 4R and Amaranth. Slopes of 51 mV/pH and 58 mV/pH suggest that the number of transferred electrons is equal to the number of protons involved in the electrode reaction.

3.3.2. Effect of accumulation parameters

The efficiency of the EGPE to Ponceau 4R and Amaranth was related to time and potential in the accumulation process. As shown in Fig. 3C, the oxidation peak currents of Ponceau 4R and Amaranth increased slightly as the potential changed from 0.2 V to 0.4 V, but then decreased significantly over the potential range 0.4–0.7 V. The maximum response peak currents were obtained at 0.4 V. Therefore, 0.4 V was chosen as the optimal accumulation condition for the simultaneous determination Ponceau 4R and Amaranth.

Fig. 3D depicts the influence of accumulation time on the oxidation peak currents of Ponceau 4R and Amaranth. With increasing incubation time, from 0 s to 600 s, peak currents for Ponceau 4R and Amaranth showed increasing responses up to 400 s. Longer accumulation time did not obviously improve the response, suggesting the mounts of Ponceau 4R and Amaranth tend to saturated adsorptions at the EGPE surface. Thus, 400 s was selected as the optimal accumulation time for the simultaneous determination of Ponceau 4R and Amaranth.

3.4. Analytical properties

3.4.1. Reproducibility, stability and reusability

The reproducibility of the EGPE was examined using a solution containing $1.0 \times 10^{-6}$ mol/L Ponceau 4R and Amaranth, and the relative standard deviations (RSDs) of current signals, using six independently prepared EGPEs, were 4.08% and 4.62%, respectively. When the EGPE was not in use, it was stored at 25 °C. 94.7% and 94.2% of the initial response of the EGPE for Ponceau 4R and Amaranth were remained, respectively, after three weeks, when the electrodes were used just once in seven days. This result indicated the EGPE had good stability. The regeneration of the EGPE was developed by cycling voltammetry from 0.3 to 1 V 10 times in 0.05 mol/L pH 7 BR buffer solution. The renewed EGPE could restore 98.8% and 95.4% of the initial value for Ponceau 4R and Amaranth, respectively.
and Amaranth, respectively, after six assay runs. This result indicated the EGPE showed high reusability.

3.4.2. Interference

The interferences of ingredients or food additives such as sweeteners, preservatives and coloring agents were investigated at the EGPE for the simultaneous determination of Ponceau 4R and Amaranth. The peak of the Peak currents of Amaranth and Ponceau 4R at the EGPE increased with the increasing concentrations of Ponceau 4R and Amaranth, respectively, after six assay runs. This result indicates the EGPE can be successfully used for the simultaneous detection of Ponceau 4R and Amaranth in the practical application. The good performance can be attributed to high adsorbability and conductivity of the EG.

3.4.4. Sample analysis

To evaluate potential applications, the proposed method was applied to the simultaneous detection of Ponceau 4R and Amaranth in grape juice samples. Recovery testing was carried out to demonstrate the validity of the proposed method. The recoveries obtained ranged from 94.5% to 107.2% and 94.3% to 98.0%, respectively, suggesting good accuracy of the proposed method. For comparison, the concentrations of Ponceau 4R and Amaranth in these samples were also determined by HPLC and the results are shown in Table 2. The t test and F test were applied to statistically evaluate the results of the proposed method against HPLC. When the significant level is 0.05, the values of t and F are less than t critical value and F critical value, respectively. The assay results obtained by both methods showed a good agreement. Thus, the present method could satisfy the need for determination of Ponceau 4R and Amaranth in soft drinks.

4. Conclusion

A simple and novel electrochemical method based on SWSV method was designed using EGPE and successfully applied to the simultaneous determination of isomers of Ponceau 4R and Amaranth, respectively, after six assay runs. The assay results obtained ranged from 94.5% to 107.2% and 94.3% to 98.0%, respectively, suggesting good accuracy of the proposed method. For comparison, the concentrations of Ponceau 4R and Amaranth in these samples were also determined by HPLC and the results are shown in Table 2. The t test and F test were applied to statistically evaluate the results of the proposed method against HPLC. When the significant level is 0.05, the values of t and F are less than t critical value and F critical value, respectively. The assay results obtained by both methods showed a good agreement. Thus, the present method could satisfy the need for determination of Ponceau 4R and Amaranth in soft drinks.

4. Conclusion

A simple and novel electrochemical method based on SWSV method was designed using EGPE and successfully applied to the simultaneous determination of isomers of Ponceau 4R and Amaranth.

Table 1

<table>
<thead>
<tr>
<th>Interference</th>
<th>Peak current of Amaranth (µA)</th>
<th>Peak current of Ponceau 4R (µA)</th>
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<tr>
<td>No interference</td>
<td>6.57 ± 0.23</td>
<td>8.20 ± 0.17</td>
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<tr>
<td>1.0 × 10⁻³ mol/L sucrose</td>
<td>6.25 ± 0.20</td>
<td>8.50 ± 0.12</td>
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<td>1.0 × 10⁻³ mol/L glucose</td>
<td>6.32 ± 0.62</td>
<td>8.10 ± 0.18</td>
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<td>1.0 × 10⁻³ mol/L sodium benzoate</td>
<td>6.32 ± 0.34</td>
<td>8.10 ± 0.18</td>
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<tr>
<td>1.0 × 10⁻³ mol/L glycine</td>
<td>6.56 ± 0.15</td>
<td>8.00 ± 0.21</td>
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<tr>
<td>1.0 × 10⁻³ mol/L vitamin C</td>
<td>6.26 ± 0.11</td>
<td>8.30 ± 0.31</td>
</tr>
<tr>
<td>1.0 × 10⁻³ mol/L citric acid</td>
<td>6.36 ± 0.21</td>
<td>8.50 ± 0.11</td>
</tr>
<tr>
<td>1.0 × 10⁻⁴ mol/L Ca²⁺</td>
<td>6.35 ± 0.15</td>
<td>7.98 ± 0.13</td>
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<tr>
<td>1.0 × 10⁻⁴ mol/L Mg²⁺</td>
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<tr>
<td>1.0 × 10⁻⁴ mol/L SO₄²⁻</td>
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<tr>
<td>1.0 × 10⁻⁴ mol/L Cl⁻</td>
<td>6.50 ± 0.26</td>
<td>8.30 ± 0.27</td>
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<tr>
<td>1.0 × 10⁻⁴ mol/L Triton X-100</td>
<td>6.20 ± 0.26</td>
<td>8.10 ± 0.17</td>
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<tr>
<td>1.0 × 10⁻⁵ mol/L Tartrazine</td>
<td>6.21 ± 0.29</td>
<td>7.89 ± 0.23</td>
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Table 2

<table>
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<tr>
<th>Sample</th>
<th>Added (µM)</th>
<th>The proposed method (µM)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>Determined by HPLC (µM)</th>
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<tr>
<td></td>
<td>Amaranth</td>
<td>Ponceau 4R</td>
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<td>1</td>
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<td>0.98</td>
<td>1.46</td>
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<td>2</td>
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<td>3</td>
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<td>6</td>
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</tr>
<tr>
<td>7</td>
<td>3.00</td>
<td>5.00</td>
<td>2.83</td>
<td>3.55</td>
<td>3.32</td>
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</table>

* Values reported are mean of three replicates.
Amaranth. The EGPE was prepared by mixing EG with solid paraffin at a mass ratio of 99:1. Due to the multiporous structure and large specific surface area of the EG, the EGPE showed enhanced electrocatalytic activity toward the oxidations of Ponceau 4R and Amaranth. Based on the electrocatalysis activity, an electrochemical method was constructed for simultaneous detection of Ponceau 4R and Amaranth by the EGPE. The proposed method for simultaneous detection of Ponceau 4R and Amaranth had a wide linear range, a low detection limit, acceptable fabrication reproducibility and stability, and excellent reusability, and was successfully applied for simultaneous detection of Ponceau 4R and Amaranth in soft drinks. This method may provide a useful tool for the simultaneous determination of the isomers of Ponceau 4R and Amaranth, and could be readily extended toward the on-site monitoring of the hazard components in food.

Acknowledgements

The authors are grateful to the financial support from the National Science Foundation of China (Nos. 21105006; 21175016), the Natural Science Foundation of Jiangsu Province, China (No. BK2011239), Natural Science Foundation of Changzhou (CM20122010) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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