

## Determination of sunset yellow and tartrazine using silver and poly (L-cysteine) composite film modified glassy carbon electrode

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Received 26 May 2015; revised and accepted 11 February 2016

Silver and poly(L-cysteine) composite film modified glassy carbon electrode (PLC/Ag/GCE) has been fabricated via cyclic voltammetry and used for investigation of the electrochemical behavior of sunset yellow (SY) and tartrazine (TT). A pair of anodic peak at 0.760 V (vs. Ag/AgCl) and cathodic peak at 0.701 V (vs. Ag/AgCl) for SY and an anodic peak at 1.013 V (vs. Ag/AgCl) of TT are observed in pH 4.5 phosphate buffer solution. Based on the two well-resolved anodic peaks of SY and TT, a novel electrochemical method has been successfully developed for simultaneous determination of SY and TT using differential pulse voltammetry. Under the optimized experimental conditions, the linear range for the determination of SY and TT are  $5.00 \times 10^{-7}$ – $3.00 \times 10^{-4}$  mol L<sup>-1</sup> and  $7.50 \times 10^{-7}$ – $7.50 \times 10^{-4}$  mol L<sup>-1</sup>, respectively with detection limits of  $7.50 \times 10^{-8}$  mol L<sup>-1</sup> and  $2.50 \times 10^{-7}$  mol L<sup>-1</sup>, respectively. The proposed method has been applied for simultaneous determination SY and TT in beverage with satisfactory results.

**Keywords:** Analytical chemistry, Electroanalytical methods, Electrodes, Modified electrodes, Films, Composite films, Sunset yellow, Tartrazine

As an important food additive, edible synthetic colorants help in improving the appearance, color and texture of food and drinks, such as candy, fruit juice, carbonated beverage, etc. Unfortunately, synthetic dyes usually contain azo (N=N) functional groups and aromatic ring structures, which are harmful to human health<sup>1</sup>. Hence, it is important to monitor and control the usage of the edible synthetic colorant in high consumption products. Currently, among the synthetic dyes, sunset yellow (SY) and tartrazine (TT) are the two most widely used synthetic pigments. Thus, developing a stable, sensitive and selective sensor for SY and TT has considerable significance for food inspection and guiding the dosage of edible synthetic colorant. Recently, many methods have been used to determine SY and TT, such as spectrophotometry<sup>2-5</sup>, electrochemical method<sup>6-9</sup>, high-performance liquid

chromatography<sup>10,11</sup>, thin layer chromatography<sup>12</sup>, photoacoustic spectroscopy<sup>13</sup>, etc. Due to the high sensitivity and simple operations, electrochemical method has achieved great development in the past several years. Ghoreishi *et al.*<sup>14</sup> used gold nanoparticles carbon paste electrode to determine SY and TT; however, the linear range of their method was narrow. The glassy carbon electrode modified with metal silver is reported to improve the electrical conductivity of electrode and enhance the response signal to As(III) and Cr(VI)<sup>15,16</sup>. Poly(L-methionine) modified glassy carbon electrode has realized simultaneous determination of acid, xanthine and hypoxanthine in human serum samples<sup>17</sup>. This illustrates that poly(amino acid) modified electrodes have a good catalytic effect on some organic molecules. Herein, silver and poly(L-cysteine) composite film modified electrode (PLC/Ag/GCE) has been prepared. The polymerized layer of L-cysteine covering the metallic silver reduces the dissolution loss of silver, because of which the stability of the electrode is improved. Moreover, PLC/Ag/GCE exhibits good sensitivity and precision toward SY and TT due to the synergistic effects of silver and poly (L-cysteine). Under the optimal conditions, a new method for simultaneous determination of SY and TT has been established and successfully applied to analyze beverage samples.

### Experimental

Electrochemical experiments were conducted with a Zennium electrochemical workstation (Zahner, Germany). A three-electrode system was used for all electrochemical experiments, which consisted of an Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as the auxiliary electrode, a bare GCE (diameter 3.0 mm) or a modified electrode as working electrode. The surface morphology of electrode was observed with a scanning electron microscope (JEOL JSM-6610LV, Japan). All pH measurements were made on a PHS-3C digital pH meter (Shanghai Leici Device Works, China).

Sunset yellow, tartrazine, L-cysteine and silver nitrate were obtained from Sigma-Aldrich. All other chemicals used were of analytical grade. Doubly distilled water was used throughout the experiments.

Phosphate buffer solutions (PBS) were prepared with  $0.1 \text{ mol L}^{-1} \text{ H}_3\text{PO}_4\text{-Na}_3\text{PO}_4$  and used as supporting electrolyte. All standard solutions and buffer solutions were stored in a refrigerator at  $4 \text{ }^\circ\text{C}$ , while all experiments were carried out at room temperature.

Before surface modification, a bare GCE was polished with  $0.05 \text{ }\mu\text{m}$   $\alpha$ -alumina powder, until a mirror-like finish was obtained, and then sonicated with 1:1  $\text{HNO}_3$  solution, ethanol, and doubly distilled water in an ultrasonic aqueous bath for 5 min in sequence. The treated GCE was cyclic scanned from  $-0.9$  to  $-0.1 \text{ V}$  at a scan rate of  $0.08 \text{ V s}^{-1}$  for 8 cycles in a mixture containing  $3.0 \times 10^{-3} \text{ mol L}^{-1} \text{ AgNO}_3$  and  $0.5 \text{ mol L}^{-1} \text{ HNO}_3$ . After modifying the surface of bare GCE with silver, the modified electrode (Ag/GCE) was electropolymerized by sweeping from  $2.4$  to  $-0.8 \text{ V}$  at a scan rate of  $0.14 \text{ V}\cdot\text{s}^{-1}$  for another 8 cycles in a solution with PBS ( $\text{pH} = 4.5$ ) and  $5.00 \times 10^{-3} \text{ mol L}^{-1}$  L-cysteine. After polymerization, the electrode was removed, rinsed thoroughly with doubly distilled water and air-dried. Finally, silver and poly(L-cysteine) composite film modified electrode (PLC/Ag/GCE) was successfully obtained.

## Results and discussion

Figure 1 shows the SEM images of the surface of GCE, PLC/GCE, Ag/GCE and PLC/Ag/GCE. The bare GCE and PLC/GCE presents a smooth surface, while Ag/GCE has a rough and dotted surface. After the electrochemical polymerization of L-cysteine on Ag/GCE, PLC/Ag/GCE displays a roughness-lowered and dot-reduced surface. On observing the electrodes with naked eye, it is seen that Ag/GCE is covered with a silver-white film, while PLC/GCE and PLC/Ag/GCE have colored films. These details indicate that silver ions have been deposited and form metallic silver on bare GCE, while L-cysteine polymer is formed on both bare GCE and Ag/GCE.

Electrochemical impedance spectroscopy (EIS) is a valid method to characterize the features of a surface-modified electrode and can give information on impedance changes of the electrode surface during the modification process. The semicircle diameter at higher frequencies corresponds to the electron-transfer resistance ( $R_{ct}$ ), while the linear part at lower frequencies is related to the diffusion process<sup>18</sup>. Figure 2 depicts the Nyquist plots of the EIS at GCE, PLC/GCE, Ag/GCE and PLC/Ag/GCE in the presence

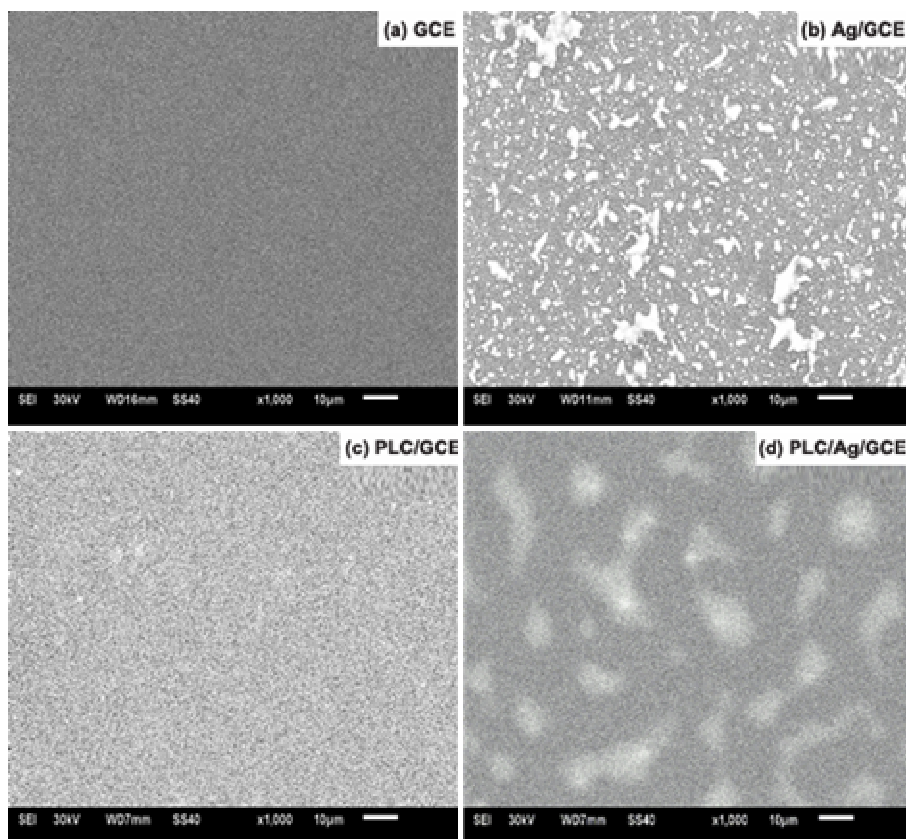


Fig. 1 — SEM images of (a) GCE, (b) Ag/GCE, (c) PLC/GCE and (d) PLC/Ag/GCE.

of  $1.0 \times 10^{-3} \text{ mol L}^{-1} \text{ K}_3[\text{Fe}(\text{CN})_6]$  and  $0.1 \text{ mol L}^{-1} \text{ KCl}$  solution. The inset of Fig. 2 is the Randles circuit model; their corresponding  $R_{ct}$  value were calculated to be  $1051 \Omega$ ,  $210.8 \Omega$ ,  $90.76 \Omega$  and  $168.6 \Omega$ , respectively. The EIS results demonstrate that due to the PLC, Ag and PLC/Ag films, electrons can speed through the interface between electrode and probe molecules as compared with through a bare GCE, especially Ag/GCE.

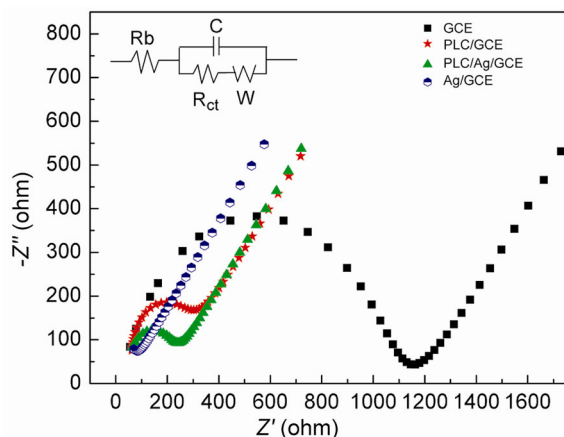


Fig. 2 — Electrochemical impedance spectra of bare GCE, Ag/GCE, PLC/GCE and PLC/Ag/GCE.

[Inset is equivalent electrical circuit diagrams for impedance plots. Rb: bulk resistance,  $R_{ct}$ : charge transfer resistance, C: double-layer capacitance, W: Warburg resistance].

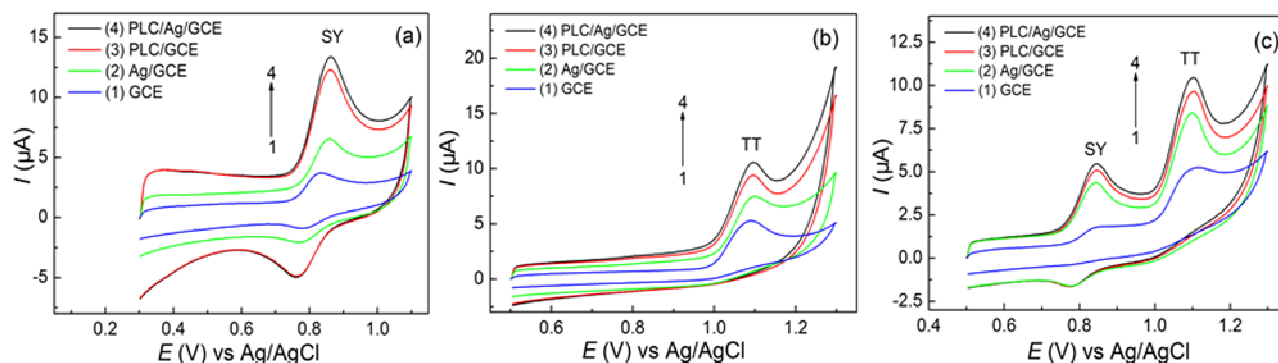


Fig. 3 — Cyclic voltammograms of (a) SY, (b) TT, and, (c) their mixed solution at GCE (1), PLC/GCE (2), Ag/GCE (3) and PLC/Ag/GCE (4).

Figure 3 shows CV recordings of  $5.00 \times 10^{-5} \text{ mol L}^{-1}$  SY ( $pH$  2.5),  $1.00 \times 10^{-4} \text{ mol L}^{-1}$  TT ( $pH$  4.5) and their mixture ( $pH$  4.5) at bare GCE, PLC/GCE, Ag/GCE and PLC/Ag/GCE. As can be seen, a couple of redox peaks of SY and an anodic peak of TT are obtained from the cyclic voltammograms. SY and TT exhibit poor electrochemical responses at bare GCE, while their peak currents increase at PLC/GCE, Ag/GCE and PLC/Ag/GCE. For SY, the anodic peak current is stronger than the cathodic peak. Table 1 lists the related anodic peak currents and anodic potentials of SY and TT at different electrodes. PLC/Ag/GCE shows the strongest electrocatalytic capability toward SY and TT. Also, it can distinguish between the characteristic signals of the two compounds in a mixture. The metallic silver ensures a good conductivity and the structure of poly(amino acids) supplies sufficient active sites during catalysis because of its active groups such as amino group, carboxyl group, etc. Thus, the good electrocatalytic capability of PLC/Ag/GCE may be attributed to the synergistic effects of Ag and PLC.

The cyclic voltammograms of SY and TT were recorded from  $pH$  2.5 – 8.0. The results illustrate that with the value of  $pH$  rising, the anodic peak potential ( $E$ ) of SY and YY both shift to negative potential with regression equations of  $E_{(SY)} = 0.9295 - 0.03013 pH$ ,  $r = 0.9971$ , and  $E_{(TT)} = 1.228 - 0.04041 pH$ ,  $r = 0.9980$ ,

Table 1 – Anodic peak currents and anodic potentials at different electrodes

Electrode	Individual				Simultaneous			
	SY		TT		SY		TT	
	$I$ :( $\mu\text{A}$ )	$E$ :(V)	$I$ :( $\mu\text{A}$ )	$E$ :(V)	$I$ :( $\mu\text{A}$ )	$E$ :(V)	$I$ :( $\mu\text{A}$ )	$E$ :(V)
GCE	2.25	0.834	2.36	1.090	1.12	0.848	3.36	1.114
Ag/GCE	4.49	0.862	6.42	1.098	3.25	0.844	5.46	1.100
PLC/GCE	8.41	0.858	7.85	1.096	3.82	0.846	6.22	1.101
PLC/Ag/GCE	9.41	0.862	8.73	1.098	4.43	0.846	6.69	1.102

suggesting that the protons have participated in electrode reactions. The peak current of SY decline in response to a increasing pH. However, the peak current of TT increases and then decreases, the maximum value is observed at pH 4.5. In view of the satisfactory peak current and good peak separation, pH 2.5 and pH 4.5 were used in individual determination for SY and TT, respectively, and pH 4.5 was selected as an optimum condition for the simultaneous determination of these two compounds. Herein, based on the general Nernst equation and the slopes of 0.03013 (SY) and 0.04041 (TT) in  $E_p$  vs pH plots (the slopes for both were between 0.059/2 and 0.059) it may be theoretically considered that in both case (SY or TT) two-electron transfer occurs during the electrode reactions.

The cyclic voltammograms at various scan rates were performed at PLC/Ag/GCE in the presence of  $5.00 \times 10^{-5}$  mol L<sup>-1</sup> SY and  $1.00 \times 10^{-4}$  mol L<sup>-1</sup> TT, respectively. With the increase of the scan rate in the range of 0.04 ~ 0.40 V s<sup>-1</sup>, the anodic peak current of SY increased gradually and the logarithm of the anodic peak current versus the logarithm of scan rate fits linear relationship with the regression equations of  $\lg I (\mu\text{A}) = -0.08672 + 0.8331 \lg v (\text{V s}^{-1})$ ,  $r = 0.9901$ . The slope value of 0.8331 indicates that the redox of SY is jointly controlled by diffusion and adsorption, but mainly adsorption. In the case of TT, the anodic peak current and the square root of scan rate obeys the regression equation  $I (\mu\text{A}) = 0.8144 + 1.214 v^{1/2}$ ,  $r = 0.9933$ , which demonstrates that the electro-oxidation of TT is a diffusion controlled process. The adsorbed amount of SY on the surface of PLC/Ag/GCE and the diffusion coefficient of TT was further estimated using Eq. (1) and Eq. (2), respectively.

$$i_p = \frac{n^2 F^2 \Gamma A v}{4 RT} \quad \dots(1)$$

$$i_d = \frac{n F A D^{1/2} C_0}{(\pi t)^{1/2}} \quad \dots(2)$$

Herein,  $n$  is the number of electrons involved in the reaction,  $\Gamma$  is adsorbed amount,  $A$  is the surface area of the modified electrode,  $v$  is scan rate,  $R$  is gas constant,  $T$  is thermodynamic temperature,  $D$  is diffusion coefficient,  $C_0$  is concentration,  $i_d$  and  $i_p$  are the peak current,  $t$  is time and  $F$  is Faraday constant. Since two electrons participate in the electrode reaction of SY, as well as TT, the adsorbed amount of

SY was calculated to be  $4.95 \times 10^{-10}$  mol cm<sup>-2</sup> in PBS (pH 2.5) at a scan rate of 0.12 V s<sup>-1</sup>. Based on chronoamperometry data of  $1.00 \times 10^{-4}$  mol L<sup>-1</sup> TT (Fig. 4), the diffusion coefficients at bare GCE and PLC/Ag/GCE were calculated as  $1.861 \times 10^{-5}$  cm<sup>2</sup> s<sup>-1</sup> and  $5.481 \times 10^{-5}$  cm<sup>2</sup> s<sup>-1</sup>, respectively. The  $D$  value of PLC/Ag/GCE is higher than that of bare GCE, implying that the modification of PLC/Ag film changes the environment of diffusion layer and accelerates the diffusion of TT.

Since differential pulse voltammetry (DPV) has higher current sensitivity and better resolution than CV<sup>19</sup>, it was used for simultaneous determination of SY and TT. The DPV curves of SY, TT and their mixture solution at various concentrations is shown in Fig. 5, while Table 2 lists their linear ranges and detection limits. In the present manuscript, all DPV experiments were carried out using 0.005 V step height, 0.04 V pulse height, 0.04 s pulse width, 0.2 s step width, 2 min time of integration.

The reproducibility of PLC/Ag/GCE was successively measured twenty times by assaying the mixed solution of  $5.00 \times 10^{-5}$  mol L<sup>-1</sup> SY and  $1.00 \times 10^{-4}$  mol L<sup>-1</sup> TT. The relative standard deviation (RSD) of 2.7% and 2.3% were obtained respectively, implying remarkable reproducibility. To ensure stability, PLC/Ag/GCE was stored dry in a refrigerator (4 °C) when not in use. The results showed that after 10 days, the DPV responses of PLC/Ag/GCE to  $5.00 \times 10^{-5}$  mol L<sup>-1</sup> SY and  $1.00 \times 10^{-4}$  mol L<sup>-1</sup> TT changed little in comparison with the initial response, suggesting an adequate stability.

In order to investigate the selectivity of PLC/Ag/GCE, the determination of  $5.00 \times 10^{-5}$  mol L<sup>-1</sup> SY and  $1.0 \times 10^{-4}$  mol L<sup>-1</sup> TT was studied in the

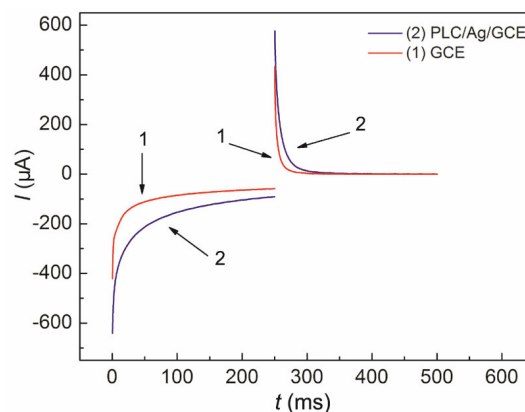


Fig. 4 — Chronoamperograms of TT at GCE (1) and PLC/Ag/GCE (2) in PBS (pH 4.5).

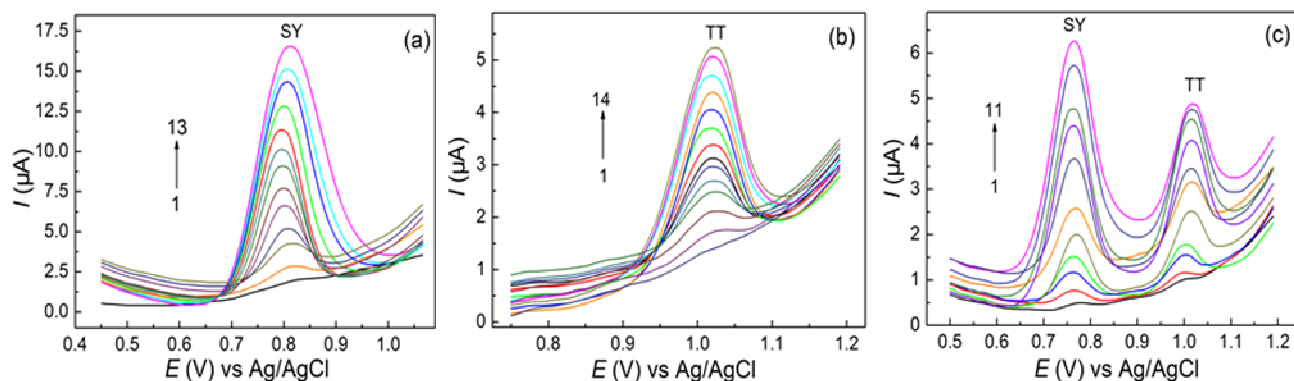


Fig. 5 — Differential pulse voltammetric curves of varying concentrations ( $\mu\text{mol L}^{-1}$ ) of (a) SY (1–13: 0.10, 0.25, 0.50, 1.00, 2.50, 5.00, 7.50, 10.0, 25.0, 50.0, 75.0, 100, 150), (b) TT (1–14: 0.50, 0.75, 1.00, 2.50, 5.00, 7.50, 10.0, 25.0, 50.0, 75.0, 100, 150, 300, 500), and (c) mixed solution of SY and TT (1– 11, SY: 0.50, 0.75, 1.00, 2.50, 5.00, 7.50, 10.0, 25.0, 50.0, 75.0, 100; TT: 1.00, 5.00, 7.50, 10.0, 25.0, 50.0, 75.0, 100, 250, 500, 750).

Table 2 – Analytical parameters for determination of SY and TT at PLC/Ag/GCE

Measurement method	Analyte	Linear range ( $\text{mol L}^{-1}$ )	Linear regression equation	Corr. coeff.	Detection limit ( $\text{mol L}^{-1}$ )
Individual	SY	$1.00 \times 10^{-7} - 1.00 \times 10^{-5}$	$I = 1.568 + 7.636 \times 10^5 C$	0.9919	$5.00 \times 10^{-8}$
		$1.00 \times 10^{-5} - 1.50 \times 10^{-4}$	$I = 8.470 + 4.498 \times 10^4 C$	0.9934	
	TT	$5.00 \times 10^{-7} - 1.00 \times 10^{-5}$	$I = 0.098 + 1.373 \times 10^4 C$	0.9932	$7.50 \times 10^{-8}$
		$1.00 \times 10^{-5} - 5.00 \times 10^{-4}$	$I = 1.650 + 7.157 \times 10^3 C$	0.9949	
Simultaneous	SY	$5.00 \times 10^{-7} - 1.00 \times 10^{-5}$	$I = 0.5439 + 1.902 \times 10^5 C$	0.9907	$7.50 \times 10^{-8}$
		$1.00 \times 10^{-5} - 3.00 \times 10^{-4}$	$I = 2.583 + 9.964 \times 10^3 C$	0.9904	
	TT	$7.50 \times 10^{-7} - 7.50 \times 10^{-5}$	$I = 0.1112 + 2.597 \times 10^4 C$	0.9828	$2.50 \times 10^{-7}$
		$7.50 \times 10^{-5} - 7.50 \times 10^{-4}$	$I = 1.962 + 4.241 \times 10^2 C$	0.9951	

<sup>a</sup> $I$  ( $\mu\text{A}$ );  $C$  ( $\text{mol L}^{-1}$ ).

Table 3 – Determination of SY and TT in beverage samples<sup>a</sup>

Sample	Analyte	Recommended ( $\mu\text{g mL}^{-1}$ )	Average ( $\mu\text{g mL}^{-1}$ )	RSD (%)	Added (%)	Found ( $\mu\text{g mL}^{-1}$ )	Recovery (%)
Carbonated beverage	SY	2.50	2.31 (2.48) <sup>b</sup>	3.6 (2.8) <sup>b</sup>	2.26	4.62 (4.77) <sup>b</sup>	102.2 (101.6) <sup>b</sup>
	TT	0.75	0.81 (0.93) <sup>b</sup>	2.0 (3.3) <sup>b</sup>	2.67	3.47 (3.76) <sup>b</sup>	99.6 (104.4) <sup>b</sup>
Fruit beverage	SY	3.50	3.60 (3.47) <sup>b</sup>	2.7 (4.5) <sup>b</sup>	2.26	5.89 (5.49) <sup>b</sup>	101.3 (95.8) <sup>b</sup>
	TT	1.50	1.35 (1.48) <sup>b</sup>	4.4 (3.9) <sup>b</sup>	2.67	3.97 (4.07) <sup>b</sup>	98.7 (98.1) <sup>b</sup>

<sup>a</sup> $n = 6$ ; <sup>b</sup>Results obtained using method described in Ref. 20.

presence of some interferents. The tolerance limit was set as the maximum concentration of the foreign substances that caused an approximately  $\pm 5\%$  relative error in the determination. The results showed that  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ , oxalic acid, citric acid, glucose, starch, L-threonine, L-valine, L-tyrosine, L-histidine ( $\geq 1.0$  mg), and  $\text{Cu}^{2+}$  (0.2 mg),  $\text{Cr}^{3+}$  (0.2 mg),  $\text{Fe}^{3+}$  (0.1 mg),  $\text{Pb}^{2+}$  (0.5 mg), carmine (0.5 mg) and ascorbic acid (0.2 mg) do not interfere with the determination

of SY and TT. Therefore, PLC/Ag/GCE possesses good selectivity and can be used to determine SY and TT simultaneously.

Carbonated beverage and fruit beverage were analysed to examine the reliability of the proposed DPV method. Carbonated beverage (1.0 mL) or fruit beverage (2.5 mL) was taken in a 10 mL volumetric flask, diluted with  $0.1 \text{ mol L}^{-1}$  PBS ( $\text{pH } 4.5$ ), mixed and then poured into an electrolytic cell. Before the assay, dissolved oxygen was eliminated by bubbling

nitrogen through the solution. Each experiment was repeated six times (Table 3). The reliability of the present method was validated by analyzing SY and TT in the samples with an earlier reported electrochemical method<sup>20</sup>. These data are also listed in Table 3. The results of the two methods were very close.

In conclusion, a novel modified glassy carbon electrode has been successfully fabricated via the construction of Ag and PLC film. The electrode exhibits good conductivity and high electrocatalytic activity for estimation of SY and TT. The anodic peaks of SY and TT were well separated by applying CV and DPV. The redox reaction of SY is mainly a absorption controlled process while the oxidation of TT is a diffusion controlled process. Owing to the excellent analytical performances of PLC/Ag/GCE, such as stability, reproducibility, selectivity and sensitivity, it can be used as a useful tool for simultaneous determination of SY and TT.

#### Acknowledgement

This research was funded by Anhui Province Universities Provincial Natural Science Research Foundation for Key Project (No. KJ2011A255), China.

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