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Original Paper

Electrochemistry and voltammetric determination of tannic acid on a single-wall carbon nanotube-coated glassy carbon electrode

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Abstract. The voltammetric behavior of tannic acid (TA) on a single-wall carbon nanotubes (SWNTs) modified glassy carbon electrode has been investigated by cyclic voltammetry. TA can generate a well-defined anodic peak on the modified electrode at around 0.42 V (vs. SCE) in 0.10 M phosphate buffer solutions ($pH = 4.0$). The electrochemical reaction involves 1e transfer, accompanied by one proton. The electrode process is controlled by adsorption. The parameters affecting the response of TA, such as solution pH, accumulation time and accumulation potential are optimized for the determination of TA. Under the optimum conditions, the peak current changes linearly with the TA concentration in the range of 5.0×10^{-8}–1.0×10^{-6} M. The lowest detectable concentration of TA is 8.0×10^{-9} M after 180 s accumulation. This method has been successfully applied to the determination of TA in tea and beer samples. In addition, the influence of potential interferents is examined. In the presence of bovine serum albumin, the peak current of TA decreases linearly due to the formation of a super-molecular complex.

Key words: Tannic acid; single-wall carbon nanotubes; glassy carbon electrode; voltammetry.

Single-wall carbon nanotubes (SWNTs) consist of rolled-up tubular shells of graphite sheet that are made up of benzene-type hexagonal rings of carbon atoms. Since their discovery by Iijima they have received a great deal of interest [1]. SWNTs have many unique properties such as large surface area, high electrical conductivity, good chemical stability and significant mechanical strength, which prompt the research efforts aimed at finding practical applications for special materials. So far, SWNTs has been widely used to fabricate supercapacitors [2, 3], batteries [4], sensors [5] and modified electrodes. SWNTs based electrodes generally are prepared by casting SWNTs suspension on conventional electrode surface [6–8], mixing SWNTs with bonds to form SWNTs paste electrode [9], or mixing SWNTs with other materials to prepare composite film modified electrode [10]. The resulting electrodes have been successfully utilized in the sensitive detection of various biological molecules such as uric acid [6], folic acid [7], cytochrome c [8]. Generally, SWNTs based electrodes can enhance the detection sensitivity and improve reversibility as it can promote electron transfer [11, 12].

Tannic acid (TA) is a natural polyphenolic compound, whose structure is as shown in Fig. 1. TA is widely used in food and medicine industry. It can be used as clarifying agent in the brewing and wine industry, and as flavouring agent in baked foods, candy and meat products. It is also used as additive in medicinal products, including those used for treatment of burns, diarrhoea [13]. TA has anti-oxidant,

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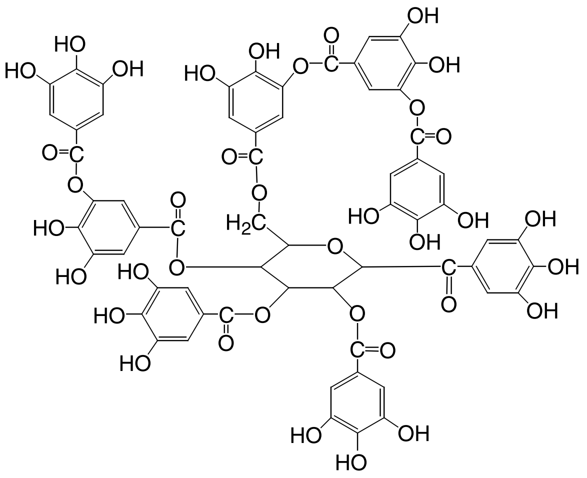

Fig. 1. Molecular structure of TA

antimutagenic and anticarcinogenic activities [14]. Therefore, the determination of TA is of some significance in food, medical and other industrial products. In general, TA is determined by protein-precipitation [15], UV spectrophotometry [16], liquid chromatography [17], etc. Since TA is electroactive, it is also studied and determined by electrochemical means. Qu and Zhou studied the electrochemical oxidation of TA by voltammetry on a glassy carbon electrode (GC) [18]. An irreversible oxidation peak at 0.31 V was observed and the detection limit they obtained was 1.3×10^{-8} M. Du and Chen established a linear sweep polarography for the indirect determination of TA in traditional Chinese medicine, which is based on the precipitation reaction of tannin with Cu^{2+} and the sensitive polargraphic wave of Cu^{2+} in NH_3–NH_4Cl medium [19]. Zhao et al. succeeded in developing a fungus biosensor for it, which is based on an oxygen electrode and aspergillus ustus immobilized in poly (vinylalcohol) in a batch system [20]. However, to our knowledge, methods based on CNTs modified electrodes have not been reported for TA.

In this work, a single-wall carbon nanotubes modified glassy carbon electrode was fabricated and used to study the electrochemistry of TA and to determine it. The new method possessed obvious advantages with regard to sensitivity, reproducibility and simplicity.

Experimental

Reagents

Tannic acid was purchased from Sinopharm Group Chemical Reagent Co. Ltd (Shanghai, China), and the stock solution (1.0×10^{-3} M) was prepared with redistilled water. The SWNTs (purity: >90%; length: 5–15 µm; external diameter: <2 nm) came from Shenzhen Nanotech Port Co. Ltd (China). Dihexadecyl hydrogen phosphate (DHP, Fluka Chemical Reagent Co., USA) was stored at −18 °C. Other reagents used were of analytical or reagent grade and water was redistilled with a fused-silica apparatus. The beer sample (brand: Blue Ribbon) was product of Blue Ribbon Group (Guangdong, China); the tea sample (brand: Tian Long) came from the Tea Company of Wuhan (China).

Apparatus

Electrochemical measurements were carried out on a CHI 660A electrochemical analytical instrument (CH Instrument Inc., USA). A three-electrode system was used, including a bare GC electrode ($\Phi = 3.0$ mm) or SWNTs modified GC electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode and a platinum wire as counter electrode. The pH values of solutions were measured with a Mettler Toledo 320-S pH meter (Shanghai, China). The spectrophotometric measurement was carried out on a UV-visible spectrophotometer (Shimadzu, Japan).

Preparation of SWNTs modified electrode

A 0.50 mg of SWNTs was dispersed in 1.0 mL *N,N*-dimethylformamide (DMF) with the aid of ultrasonic agitation to prepare 0.50 mg mL^{-1} black suspension. The GC electrode ($\Phi = 3$ mm) was carefully polished to a mirror-like surface with 0.05 µm alumina slurry on a polishing pad, and then rinsed with water, ultrasonicated in ethanol and redistilled water for a few minutes. To fabricate SWNTs modified glassy carbon electrode (SWNTs/GC), 10 µL of 0.50 mg mL^{-1} SWNTs suspension was transferred on the cleaned GC electrode surface and then let the solvent evaporate under an infrared lamp. SWNTs-DHP/GC, TiO_2-SWNTs/GC and Nano-Au-SWNTs/GC modified electrodes were prepared through similar procedure.

Experimental procedure

The three-electrode system was immersed in a 10 mL cell containing an appropriate amount of TA and 0.10 M phosphate buffer solution (PBS, pH = 4.0). After accumulating for 180 s on open circuit under stirring, followed by rest for 5 s, the potential scan was initiated and cyclic voltammogram was recorded between 0.0 and +0.9 V at scan rate of 100 mV s^{-1}. Then the modified electrode underwent successively potential scan in a blank solution for 10 cycles for reuse. Similar procedure was carried out in sample determination and a standard addition method was adopted to evaluate the determination results. All measurements were carried out at room temperature.

Results and discussion

Voltammetric response of TA on SWNTs/GC

The typical voltammograms of TA on various electrodes are shown in Fig. 2. TA can produce an oxidation peak at 0.398 V on a bare GC electrode. But the peak is small, which is consistent with that reported in literature [18]. However, at the SWNTs/GC, the peak

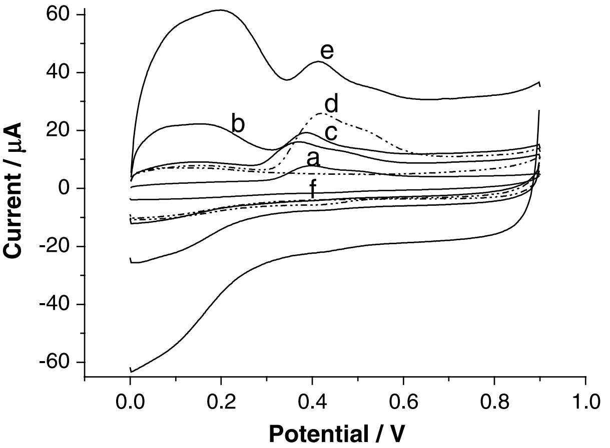

Fig. 2. Voltammograms of TA at GC (*a*), SWNTs-TiO_2/GC (*b*), SWNTs-DHP/GC (*c*), SWNTs-Nano-Au/GC (*e*), and SWNTs/GC (*d*, *f*). Solution composition: 0.10 M PBS (pH = 4.0) (*f*), or 0.10 M PBS (pH = 4.0) plus 1.0×10^{-6} M TA (*a*, *b*, *c*, *d*, *e*); scan rate: 100 mV s^{-1}; accumulation time: 180 s (on open circuit); volume of SWNTs suspension: 10 μL

becomes large markedly. The peak current is about four times as large as that on the bare GC electrode. This generally can be attributed to the accumulation function of SWNTs and its promotion effect on electron-transfer reaction. In addition, the active surface of the electrode increases when it is modified with SWNTs film, which also contributes to the increase in peak current. For comparison, other modified electrodes concerned SWNTs are also tested. As a result, TA can also produce an oxidation peak at SWNTs-DHP/GC, TiO_2-SWNTs/GC and Nano-Au-SWNTs/GC electrodes (Fig. 2). When the electrode is coated by the mixture of SWNTs and DHP, the peak current of TA decreases. It is thought that DHP may inhibit the heterogeneous electron transfer. In the presence of nano-TiO_2 or nano-Au, the peak current of TA decreases and the background current increases remarkably. This is related to the increase of film thickness and resistance of electron-transfer. DHP, nano-TiO_2 and nano-Au cannot improve the response of TA further. Therefore these composite film modified electrodes are not suitable for the determination of TA, and the SWNTs/GC is adopted in this work. As can be seen, TA does not exhibit any reduction peaks in the potential range, meaning the electrochemical reaction is irreversible.

Influence of pH

The variation of peak potential and peak current with pH is shown in Fig. 3. Within the range of 1.5–7.0, the peak current increases gradually until attaining a maximum at about pH 4.0, and then decreases. When pH is above 7.0, the oxidation peak almost disappears. It is related to the proton taking part in the electrochemical reaction. In basic solutions the reaction becomes difficult because of the short of proton. The peak potential shifts negatively with increasing pH, and they follow such equation: $E_{pa}(V) = 0.663 - 0.0628$ pH ($r = 0.998$). The slope is 63 mV/pH, indicating that the proton number involved equals to the electron-transfer number in the electrochemical reaction.

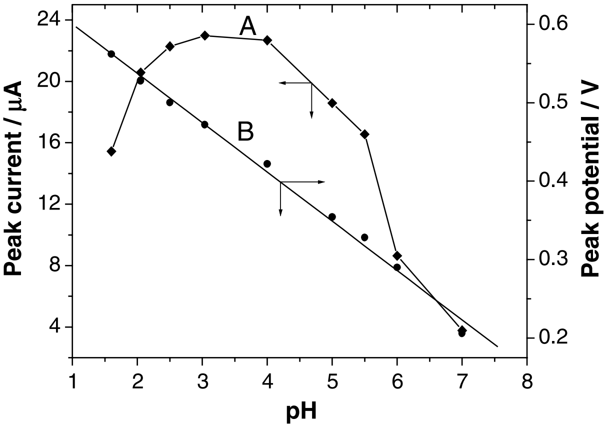

Fig. 3. Influence of pH on peak current (*A*) and peak potential (*B*) of TA. Other conditions as in Fig. 2

Influences of accumulation time and accumulation potential

As shown in Fig. 4, with increase of accumulation time, the peak current increases rapidly until it be-

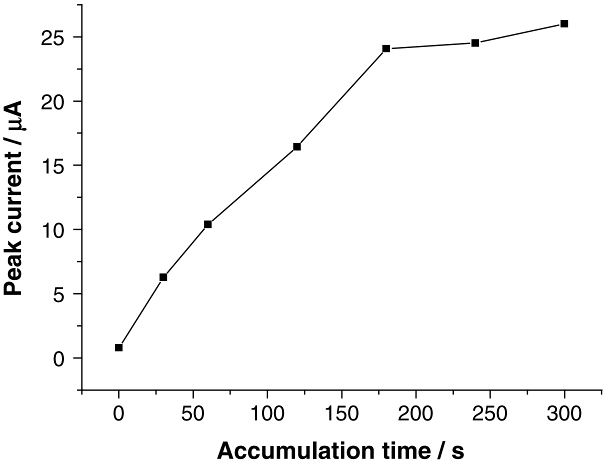

Fig. 4. Variation of anodic peak current with accumulation time. Other conditions as in Fig. 2

comes relatively stable at around 180 s, after which it changes slightly. This reflects the adsorption-controlled feature of the electrochemical process. When the accumulation potential is changed from −0.8 to +0.2 V, the peak current keeps almost unchanged, indicating that the accumulation is almost independent of electrostatic attraction. This is in agreement with the neutral nature of TA in such pH circumstance. As TA and the groups on SWNTs (i.e. –COOH, –OH) are hydrophilic, the accumulation of TA on SWNTs/GC should be ascribed to the molecular interaction between them, such as hydrogen bond. Herein, an accumulation for 180 s under open circuit is adopted.

Adsorption amount

In order to evaluate the accumulation amount of TA on the SWNTs/GC surface, the adsorption amount (Γ) was measured. Under the selected conditions, the anodic peak area increases with TA concentration rising (Fig. 5), and they show a linear relationship in the range from 0.05 to 2.5 μM. The regression equation is: $A = 1.175 + 13.34$ c (A: μC, c: μM, $r = 0.991$). When TA concentration is increased further, the increase rate of peak area lowers and at last the peak area keeps almost unchanged. This indicates that the adsorption of TA on the SWNTs/GC basically belongs to Langmuir adsorption. When TA concentration exceeds 15 μM the peak area reaches the maximum, and it is about 63.69 μC. The effective area of the SWNTs/GC electrode is estimated to be 0.101 cm^2, based on its cyclic voltammograms in a 2 mM $K_3Fe(CN)_6$ solution [21]. According to the equation $\Gamma = Q/nFA$, the Γ is calculated and it is 6.53×10^{-9} mol cm^{-2}, meaning the adsorption of TA is quite strong at the electrode.

Influence of scan rate

Peak current and scan rate (v) show a good linear relationship over the range from 20 mV s^{-1} to 450 mV s^{-1} (Fig. 6a). The regression equation is $i_{pa} = 3.88 + 107.6v$ (i_{pa} : μA, v : V s^{-1}, $r = 0.997$). This indicates that the electrochemical process of TA is controlled by adsorption. According to the equation: $i_{pa} = n^2F^2vA\Gamma_0^*/4RT = nFQv/4RT$ [22], the number of electron transferred is calculated and it is 1. This is in accordance with that reported in literature [18]. At

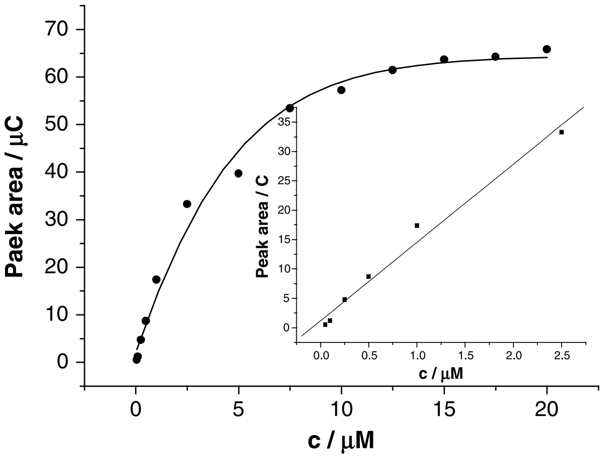

Fig. 5. Dependence of peak area on TA concentration. Inset is the corresponding plot for the range of 0.05–2.5 μM TA; other conditions as in Fig. 2

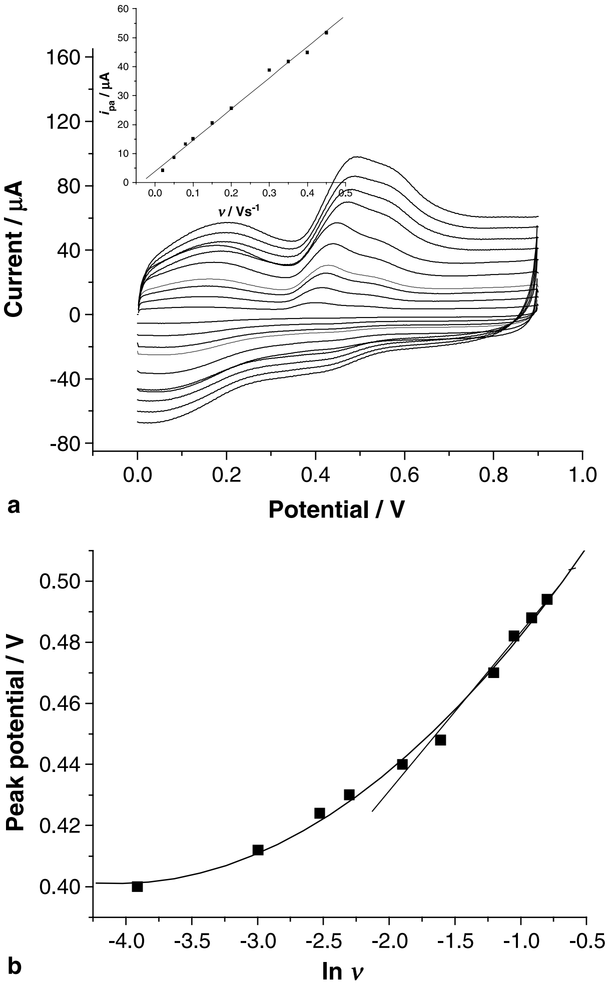

Fig. 6. Dependence of peak current (a) and peak potential (b) on scan rate. Scan rate: 20, 50, 80, 100, 150, 200, 300, 350, 400, 450 mV s^{-1} (from inner to outer); inset is the corresponding plot of peak current against scan rate; other conditions as in Fig. 2

lower scan rate, the anodic peak shifts in positive direction slowly with scan rate rising. At higher scan rate, it shifts more rapidly. Further more, the peak potential and the logarithm of v show a linear relationship, and the regression equation is $E_{\mathrm{pa}} = 0.5407 + 0.05767 \ln v$ (E_{pa}: V, v: V s^{-1}, $r = 0.999$) (Fig. 6b). For an adsorption-controlled system, the peak potential and $\ln v$ should obey the equation [23]: $E_{\mathrm{pa}} = E\deg' + m[0.78 + \ln(D^{1/2}\ k_{\mathrm{s}}{}^{-1}) - \ln m] + m \ln v$ (where $m = RT/[(1\text{-}\alpha)\ n_{\alpha}F]$). Hence the electron transfer coefficient α can be estimated and it is 0.55.

Calibration curve and stability

The differential pulse voltammograms of TA are illustrated in Fig. 7. Under the optimized conditions, the anodic peak current is linear to TA concentration over the range of 5.0×10^{-8}–1.0×10^{-6} M. The regression equation is: $i_{\mathrm{pa}} = 0.17 + 23.7$ c (i_{pa}: µA, c: µM, $r = 0.998$). When the concentration is increased further, the $\mathrm{i_{pa}}$ vs. c curve deviates from the plot, resulting from the decrease of accumulation efficiency. The lowest detectable concentration of TA is estimated to be 8.0×10^{-9} M after 180 s accumulation. This indicates that the method is quite sensitive. After every measurement the SWNTs/GC is transferred in a blank solution to regenerate through successive potential scanning between 0 and 0.9 V for several times. As an example, a 1.0×10^{-6} M TA solution is determined for eight times with an electrode regenerated after every measurement, the relative standard deviation (RSD) for the anodic peak current is 4.8%. The electrode is used daily and stored in the air. After several days of repetitive use, the peak current changes slightly. This suggests that the SWNTs/GC electrode has good stability towards the determination of TA.

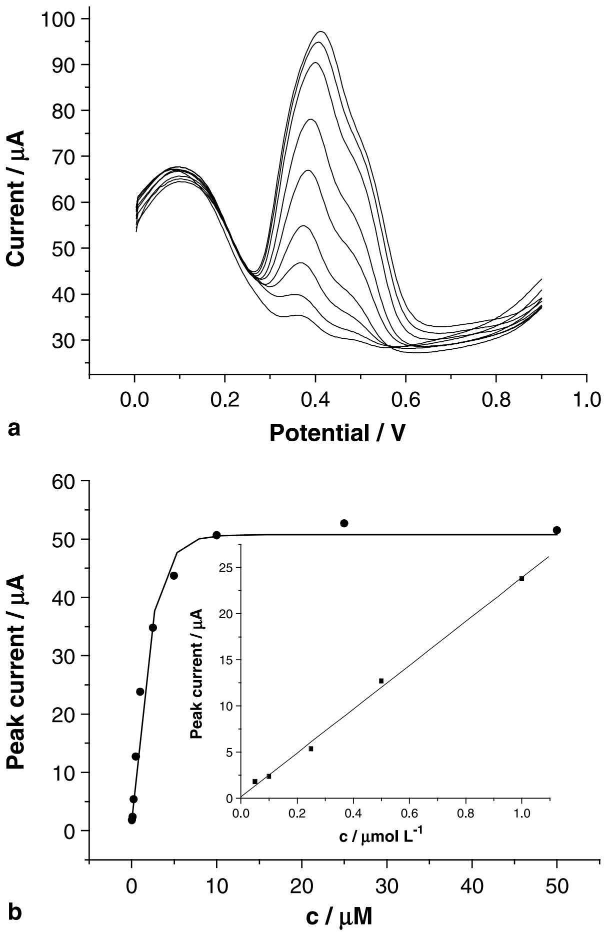

Fig. 7. Differential pulse voltammograms of TA (a) and dependence of peak current on TA concentration (b). TA concentration: 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 25, 50 µM (from inner to outer for a); insert is the corresponding plot for the range of 0.05–1 µM (b); other conditions as in Fig. 2

Interference of coexistent species

The influences of various foreign species on the determination of TA were examined. The tolerable limit of the foreign species was taken when the relative error was not greater than ±10% in the recovery. It was found that the tolerable concentration ratios with respect to 1.0×10^{-6} M TA were 500-fold for $\mathrm{Mn^{2+}}$; 200-fold for $\mathrm{K^{+}}$, $\mathrm{Na^{+}}$, $\mathrm{Mg^{2+}}$, $\mathrm{Cl^{-}}$; 100-fold for $\mathrm{Zn^{2+}}$, $\mathrm{Cu^{2+}}$, $\mathrm{SO_4{}^{2-}}$; 50-fold for $\mathrm{Ag^{+}}$, $\mathrm{NO_3{}^{-}}$; 20-fold for $\mathrm{Pb^{2+}}$. In addition, in the presence of 50-fold of potassium sodium tartrate, uric acid, 20-fold of ascorbic acid, 10-fold of glucose, barbituric acid, amion acetic acid, indoleactic acid, the determination was not interfered. As to the surfactants, TritonX-100, cetyltrimethylammonium bromide (CTAB), cetylpyridinumbromid (CPB), sodium dodecylsulfate (SDS), sodium dodecyl benzene sulfonate (SDBS), didodecyldimethylammonium bromide (DDAB), and dihexadecyldimethylammonium bromide (DCAB) were tested. In the presence of 100-fold of SDS, the peak current of TA kept almost unchanged. However, the addition of 5-fold of SDBS and Triton X-100 made the anodic peak current of 1.0×10^{-6} M TA decrease by 9.5 and 9.6%, respectively. Equal amount of CPB, CTAB, DCAB and DDAB made the anodic peak current decrease by 14.6, 20.7, 32.8, and 49.7%, respectively. This is due to the competitive adsorption of those surfactants with TA on the SWNTs/GC surface.

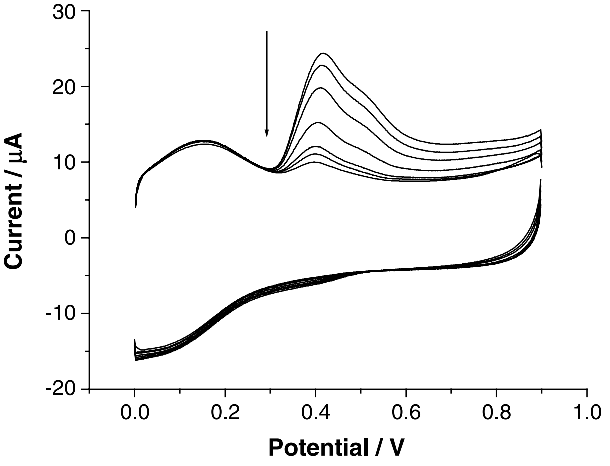

Fig. 8. Voltammograms of 1.0×10^{-6} M TA in the presence of 0, 1, 2.5, 5, 10, 20, 40 μg mL^{-1} BSA (from top to bottom). Other conditions as in Fig. 2

Influence of bovine serum albumin and DNA

Bovine serum albumin (BSA) does not exhibit observable peak at SWNTs/GC, however, it makes the oxidation peak of TA decrease (Fig. 8). Freitas et al. argued that proteins have a number of sites to which polyphenols can bind [24]. Similarly, Labieniec and Gabryelak thought polyphenols could interact with BSA and form a large network [25]. Thus, it is proposed that BSA and TA form an electroinactive complex. But no discernible change in absorption spectrum was observed to reveal their combination. The change of peak current and the BSA concentration show a linear relationship over a certain concentration range. Hence this procedure may be applied to the indirect determination and study of BSA. Similar phenomenon was observed when BAS was replaced by DNA. This was related to the strong binding of TA and DNA.

Application

This method was applied to the determination of TA in tea. Before determination 1.74 g tea-leave was taken and dissolved in heated water for one hour; after cooled down, it was filtered [26], and the filtered solution was diluted to 50 mL. Under the optimized conditions mentioned above, the sample solution was determined. Table 1 shows the measurement results, and the average recovery is 97%. According to Table 1, the TA content in the tea sample is calculated and it is 0.28%. For comparison, the sample was also determined with an UV spectrophotometry [27]. The results are shown in Table 2. The proposed method is demonstrated to be believable through *F* verification and *t* verification [28]. As to the determination of TA in beer, prior to determination the beer sample was heated to eliminate the gas dissolved in it, and then diluted to certain volume for measurement [26]. Table 3 shows the determination results, and the TA content is ca. 15.1 μg mL^{-1}, which is in accordance with that measured by using an UV spectrophotometry [29].

Table 1. Measurement results of TA in tea

No.	Add (μM)	Expected (μM)	Found (μM)	Recovery (%)	Average recovery (%)
1	0	–	0.30	–	97
2	0.30	0.602	0.583	94	
3	0.60	0.902	0.899	99	
4	0.90	1.202	1.176	97	

Table 2. Parallel measurement results of TA in tea

Time	1	2	3	4	5	6
UV (μM)	0.286	0.286	0.293	0.300	0.300	0.308
EC (μM)	0.312	0.306	0.302	0.309	0.300	0.305

Table 3. Measurement results of TA in beer

Time	1	2	3	4	Average
EC (μg mL^{-1})	15.08	15.10	15.11	15.09	15.10
UV (μg mL^{-1})	15.36	15.33	15.34	15.36	15.35

Conclusions

TA can exhibit a sensitive oxidation peak at SWNTs modified glassy carbon electrodes in pH 4.0 phosphate buffer. The electrochemical reaction involves one electron transfer, accompanied by one proton. The electrode process is adsorption-controlled and is irreversible. Under optimized conditions, the anodic peak current of TA changes linearly with its concentration in the range from 5×10^{-8}–1×10^{-6} M. The detection limit is 8.0×10^{-9} M after 180 s accumulation on open circuit. The electrode shows good stability for TA determination. This method was successfully applied to the determination of TA in tea and beer samples.

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