Bismuth Film Modified Glassy Carbon Electrode for Determination of Tannic Acid

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The electrochemical behavior of tannic acid (TA) at bismuth film coated glassy carbon electrode (Bi-GCE) has been studied by linear sweep voltammetry (LSV). Deposition of bismuth film was taken place on glassy carbon electrode (GCE) by single potential step chronoamperometric deposition at -0.45 V for 120 s. TA shows a definite cathodic peak on the Bi-GCE at about -0.6 V *vs.* Ag/AgCl in Briton Robinson (BR) buffer solution of pH 3.6 at Bi-GCE. The effects of deposition time, deposition potential, bath concentration and pH of supporting electrolyte on the reduction current of TA were optimized. Under optimum conditions, TA displayed a linear range between 0.05 μM and 200 μM, and the limit of detection (LOD) was found to be 0.035 μM. The developed electrochemical method was used for detection of TA in tea samples.

Keywords: Tannic acid, Bismuth film, Glassy carbon electrode, Linear sweep voltammetry

INTRODUCTION

As a promising substitute for the mercury film modified electrode, the bismuth film modified electrode (BiFE) has been widely used in the field of voltammetric study [1]. The BiFE shows attractive properties and excellent performance such as environmentally friendliness, high sensitivity, easy preparation, well-defined signals and negligible effect to dissolved oxygen [2-5]. According to recent experimental reports, many electrochemical analyses have been performed upon the use of BiFEs [6,7]. Toxic heavy metal ions, pharmaceutical substances, pesticides and other biological molecules and products are electrochemically determined with the BiFE [7-10].

TA is a naturally existing polyphenolic compound; its chemical structure is shown in Fig. 1. It is a water soluble natural polyphenolic compound found in various plants and fruits [11-19]. Obviously, antioxidants can hinder the oxidation of other molecules by preventing the initiation of oxidizing chain reactions and keep the human body free from the effect of reactive free radicals [20]. Like many polyphenols, TA has shown to own antioxidant [21,22], antimutagenic [23,24] and anticarcinogenic [25] properties. The most abundant antioxidant molecules in diet are phenolic compounds [26]. TA is extensively used in food and medicine industry to play an important role in the maintenance of food quality and shelf-life [27]. In brewery and wine industry, it is widely applicable as a clarifying agent, and also used as flavoring agent and additive in baked foods, candy, meat and medicinal products [28-31].

Therefore, it is of great importance to develop a suitable technique for the detection of TA in various studies. Numerous techniques have been demonstrated for the detection of TA, such as fluorescence spectroscopy [32], chromatographic techniques [33,34], capillary electrophoresis [35], electrochemiluminescence [36,37] and electrochemical methods [38-40]. However, some of the analytical methods have a certain limitations, like poor specificity and selectivity, expensive equipment and long analysis time. Hence, highly sensitive, selective, simple and cost-effective technique for the analysis of TA is very important. Due to the low cost and simplicity, electrochemical detection has gained much attention [41].

In this study, Bi-GCE was fabricated by

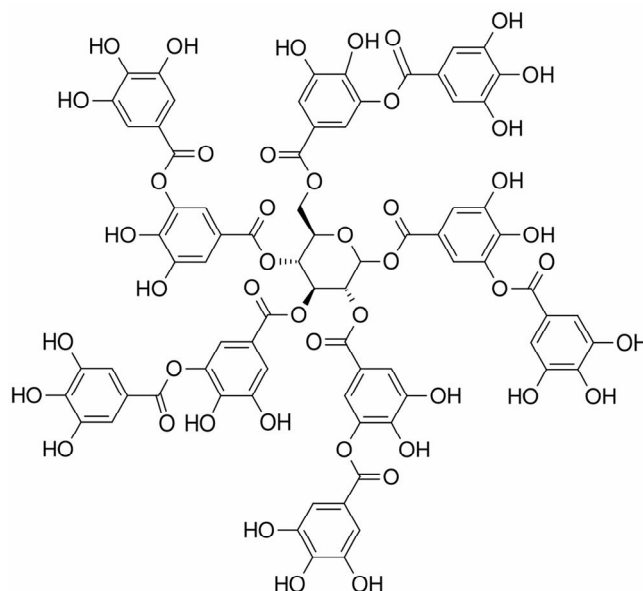*Corresponding author. E-mail: shimeles.addisu@ju.edu.et

Fig. 1. Structure of TA.

chronoamperometric method and it was found that the Bi-GCE has good voltammetric properties towards reduction of TA. At a BR buffer pH of 3.6, TA showed a well-defined reduction peak at about -0.60 V on the Bi-GCE, where the reduction peak current increased with concentration. The Bi-GCE can be used as an alternative candidate for electrochemical determination of TA because it possess a better sensitivity, reproducibility, extreme simplicity and fast response.

EXPERIMENTAL

Apparatus and Instruments

A three-electrode assembled cell was employed, consisting of Bi-GCE (3.0 mm diameter) as the working electrode, Ag/AgCl electrode as a reference electrode and a platinum wire as a counter electrode. Electrochemical techniques, such as cyclic voltammetry (CV), chronoamperometry (CA) and LSV were used using Epsilon EC-Ver 1.40.67 voltammetric analyzer (Bioanalytical Systems, USA), and electrochemical impedance spectroscopy (EIS) experiment was carried out with a CHI660E electrochemical workstation (Shanghai, China). All experiments were conducted at room temperature.

Chemicals and Reagents

TA (Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China)), bismuth nitrate ((Bi(NO$_3$)$_3$), M & B Ltd. Dagenham, England), sodium acetate (NaCH$_3$COO) (Fine, 99%), acetic acid (CH$_3$COOH) (LOBA CHEMIE, 99.5%, India), hydrochloric acid (HCl), (Riedel-de Haen, 37%, Germany), H$_3$BO$_3$ (Techno Pharmachem), NaOH (LeSOL laboratory reagent, India), H$_3$PO$_4$, 85% (Riedel de Haen, Germany). All chemicals were analytical grade chemicals.

Solution Preparation

Bi deposition bath consisting of 10 mM Bi(III) in 1 M HCl was prepared. 5 mM stock solution TA was prepared in double distilled water and other working solution of TA with the desired concentration was prepared by diluting in BR buffer solution with pH 3.6.

Electrode Cleaning and Conditioning

A GCE (3 mm in diameter) was polished with slurry of alumina (0.3 and 0.05 μm), then sonicated and washed with double distilled water. The cleanness of GCE was checked

by sweeping the potential from -1.2 V to 1.2 V in 0.1 M KCl until consistent voltammograms were obtained at a scan rate of 0.1 Vs^{-1}. The prepared electrode was used immediately after mechanical polishing and electrochemical cleaning.

Preparation of Bi-GCE

A chronoamperometric deposition of bismuth onto GCE in Bi(III) bath was taken place to prepare Bi-GCE. Briefly, a constant deposition potential of -0.45 V for 120 s was applied to the GCE in 5 mM Bi(NO$_3$)$_3$ in 1 M HCl deposition bath. The electrode was then rinsed with distilled water prepared for electroanalysis of TA.

Optimization of Experimental Parameters

Various parameters such as deposition potential, deposition time, pH of TA solution and deposition bath concentration were optimized. Plating solution containing Bi(III) in acidic media was used to avoid hydrolysis of the metal [42,43].

Optimum deposition potential was selected after examining different deposition potentials in the range of -0.25 V to -0.7 V from 5 mM of Bi(III) in 1 M HCl solution for 240 s. Deposition time was examined by changing the time of the optimum deposition potential from 45 to 360 s. Bismuth concentration, from 2 mM to 8 mM of Bi(III) in 1 M HCl, was studied as a deposition bath concentration at -0.45 V and deposition potential for 120 s. To optimize pH, all prior optimized parameters were kept constant and the pH of the solution was changed from 3 to 6. Each experimental parameter with the maximum cathodic peak current for 1 μM TA was selected.

Study of Electrode Reproducibility, Stability and Effects of Interferences

The reproducibility of the Bi-GCEs was studied by determining the same concentration of TA, 1 μM, with three electrodes prepared under the same modification conditions for three days with tree measurements in each day. Then, the percent standard deviation was calculated.

The Bi-GCE stability was determined by preparing three electrodes by keeping optimized parameters constant on the same day. The cathodic peak current of 1 μM TA was taken on the first, second and seventh day. On each day, triplicate readings were taken at the modified electrode. The average current signals of the initial day readings were compared to those of the second and seventh day.

To investigate the selectivity of Bi-GCE for the determination of TA, the effect of possible interfering substances was studied under the optimum conditions. Different interfering species such as ascorbic acid, uric acid, glucose, and VB$_1$ were added into the solution containing 1 μM of TA.

Real Sample Analysis

For real sample analysis, TA sample solution was prepared from tea. 100 ml hot water was used to extract 1.5 g of black tea sample for 10 min. The solution was then filtered and diluted to 250 ml. The collected sample solution was used for electroanalysis of TA.

RESULTS AND DISCUSSION

CV of Bi(III) Ion

CV of Bi is important for obtaining the redox potentials for Bi deposition process. The voltammogram shows some basic information, and the characteristic features of Bi reduction and oxidation. Figure 2 shows the CV of Bi deposition process. As shown in Figure 2, the oxidation and reduction of Bi was observed at about -0.058 V and -0.17 V, respectively.

CV Characterization and Determination of Active Surface Area

CV is a widely used technique for electrochemical characterization. Bare GCE and Bi-GCE were characterized using [Fe(CN)$_6$]$^{4-}$ and [Fe(CN)$_6$]$^{3-}$ redox probes. The active surface area of bare GCE and Bi-GCE was determined in 0.1 M KCl solution containing 1 mM [Fe(CN)$_6$]$^{3-}$ by recording the CVs and is shown in Fig. 3A. From the CV peak current and the diffusion coefficient of [Fe(CN)$_6$]$^{3-}$, the active surface area of the electrode was calculated by using the following equation [44],

$$i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} v^{1/2} C \ldots \ldots (1)$$

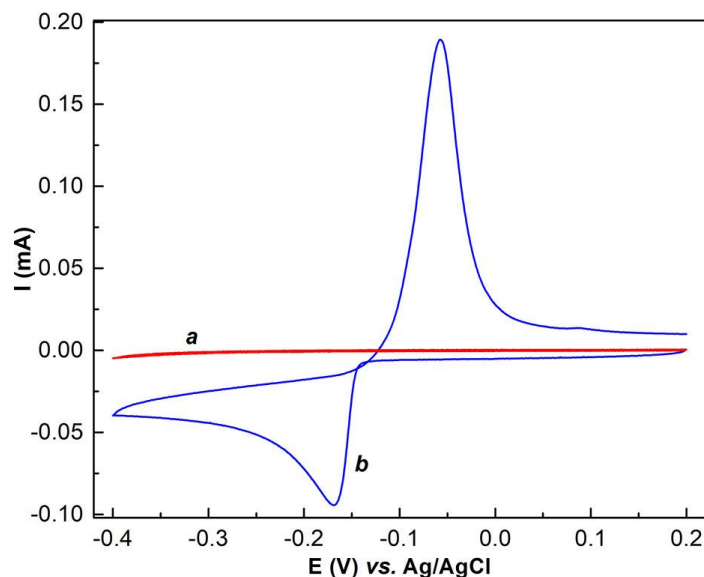where n is the number of electrons transferred, *i.e.* 1 for

Fig. 2. CVs of (a) 1 M HCl and (b) 5 mM Bi(NO$_3$)$_3$ in 1 M HCl, scan rate 0.1 V s^{-1}.

$[Fe(CN)_6]^{3-}$, A is the electrode surface area, D is the diffusion coefficient (9.382×10^{-6} cm^2 s^{-1}), the scan rate is 0.1 V s^{-1}, and C is the concentration of $[Fe(CN)_6]^{3-}$ (1×10^{-3} mol cm^{-3}). The active surface area of Bi-GCE was found to be 0.0038 cm^2, whereas the bare GCE was 0.00058 cm^2. The active surface area of Bi-GCE was about 6.5 times greater than that of the bare GCE.

Electrochemical Impedance Spectroscopy (EIS) Characterization

EIS is one the electrochemical method that can be used to examine the surface changes of the electrode in the surface modification process [45]. EIS measurements were done in 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{4-}$/1.0 mM $[Fe(CN)_6]^{3-}$ (1/1) mixture. Figure 3B shows the impedance spectrum as Nyquist plots of the bare GCE and Bi-GCE. As can be seen from Fig. 3, the diameter of the semicircular part for the Bi-GCE is smaller than bare GCE, indicating that the Bi-GCE enhanced the electron transfer between $[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$ couple and the electrode surface.

Electrochemical Behavior of TA at Bare GCE and Bi-GCE

The electrochemical behaviour of 1 µM TA was studied

using LSV in BR buffer (pH 3.6) at bare GCE and Bi-GCE. Figure 4 shows the LSVs of TA using bare GCE and Bi-GCE. The reduction of TA was observed at about -0.6 V at Bi-GCE. Comparing the bare GCE (curve C) and Bi-GCE (curve D) showed an excellent improvement in the reduction currents of TA when Bi-GCE was used. The reduction current response of Bi-GCE is about 13 times greater than the reduction current of the bare GCE.

Optimization of Parameters for Determination of TA

Optimization of deposition potential. The production of thin film by electrochemical deposition for the analysis of various analytes is usually done by forced chemical reaction. The deposition proceeds by applying an external potential. So, applied potential (deposition potential) has superposition to control the thickness of film during fabrication. For this study, deposition potential was optimized from 5 mM Bi(III) in 1 M HCl solution within a range of -0.25 V to -0.7 V, as shown in Fig. 5. The deposition potential of -0.45 V gave the highest reduction current for 1 µM TA and it was taken as an optimum deposition potential for further analysis of TA.

Optimization of deposition time. The amount of metal particles on the surface of the electrode has a great effect on
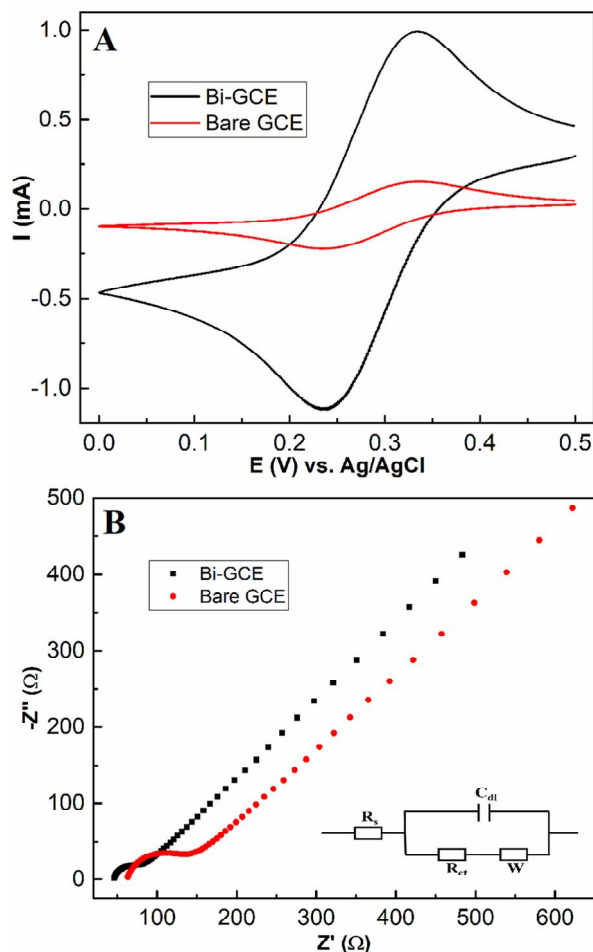
Fig. 3. (A) CVs for bare GCE and Bi-GCE, recorded at 0.1 V s^{-1} in 0.1 M KCl solution containing 1 mM K$_3$[Fe(CN)$_6$] at room temperature; (B) Nyquist plots for bare GCE the Bi-GCE recorded in 0.1 M KCl solution containing 5.0 mM [Fe(CN)$_6$]$^{4-}$/5.0 mM [Fe(CN)$_6$]$^{3-}$ (1/1) mixture. Inset: The Randle's equivalent circuit model.

the performance of the modified electrode. Therefore, the amount of metal particles on the electrode surface depends on the deposition time. So that, the deposition time should be optimized. The effect of the bismuth deposition time was investigated from 45 to 300 s. As presented in Fig. 6, the reduction peak current of TA increases with the increase in deposition time up to 120 s and remains unchanged after this time, suggesting that the bismuth layer has covered the electrode surface and further increment of thickness of the film was hindered because of the depletion of Bi(III) at interface.

Optimization of Bi-bath concentration. The influence of the bismuth bath concentration was studied from 2 mM to 8 mM Bi(III) in 1 M HCl deposited at -0.45 V for 120 s. The cathodic peak currents of 1 μM TA in BR buffer pH 3.6 increases with increasing the concentration of bismuth solution up to 5 Mm, but further increase in concentration of bismuth leads to decrease the reduction current of TA (Fig. 7), due to the growth in the bismuth film thickness. On the other hand, an increase in the concentration of Bi(III) ion is accompanied by a decrease in the reduction peak currents, because the growth of the bismuth thickness leads
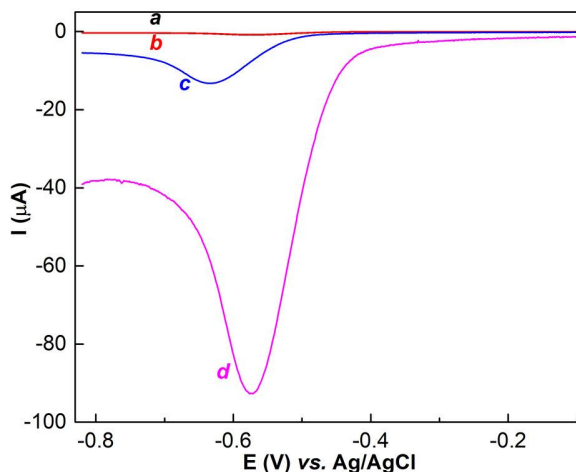
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Fig. 4. LSVs of a) bare GCE, b) Bi-GCE in BR buffer, c) bare GCE and d) Bi-GCE in 1 µM TA in BR buffer (pH = 3.6); scan rate of 0.1 V s^{-1}.
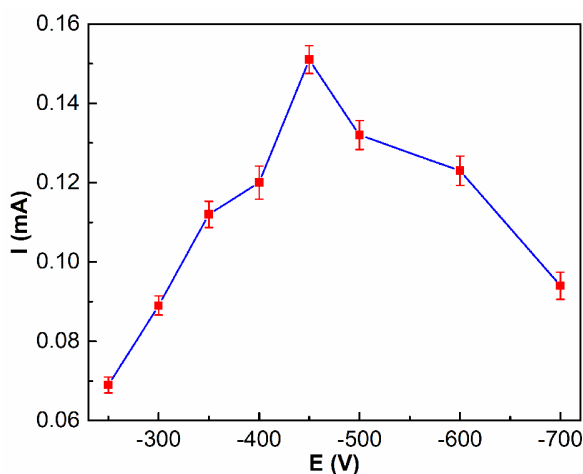

Fig. 5. The influence of deposition potential on cathodic current of 1 µM TA; 5 mM Bi(III) and 120 s deposition time.

to falling the film.

Optimization of TA pH. pH was studied in BR buffer in the range of 2-6. The results found are shown in Fig. 8A and B, in which the highest cathodic current was obtained at pH 3.6. The electrochemical reaction is related to the H$^+$ ion in the solution. The reaction is easy in acidic solution because of the presence of more H$^+$ ions. As the pH gets more basic, the H$^+$ ion begins to decline making the reaction more difficult [40]. Also, the reduction peak potential (E_{pc})

of TA shifted cathodically with the pH increase from 2 to 6, indicating that protons are involved in the electrochemical reaction. It follows a linear equation: E_{pc} = - 0.052 pH + 0.85 (R^2 = 0.972). The slope was close to the value of 0.059 V/pH, signifying that equal numbers of proton and electron participate in the electrode process. Typical polyphenols undergo 2 electron redox process in acidic medium resulting the corresponding quinones [46,47]. So,
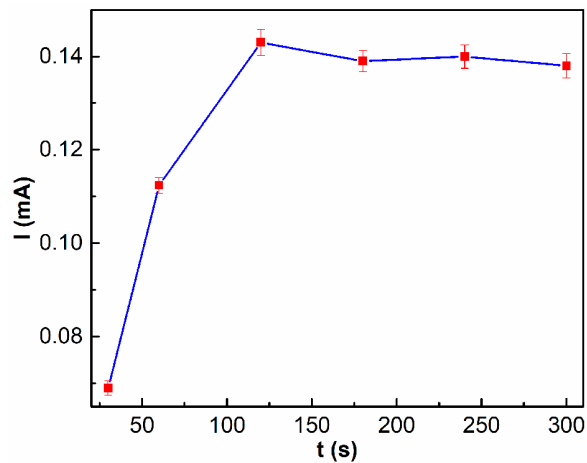
Fig. 6. The influence of deposition time on cathodic current of 1 µM TA; 5 mM Bi(III) and -450 mV deposition potential.
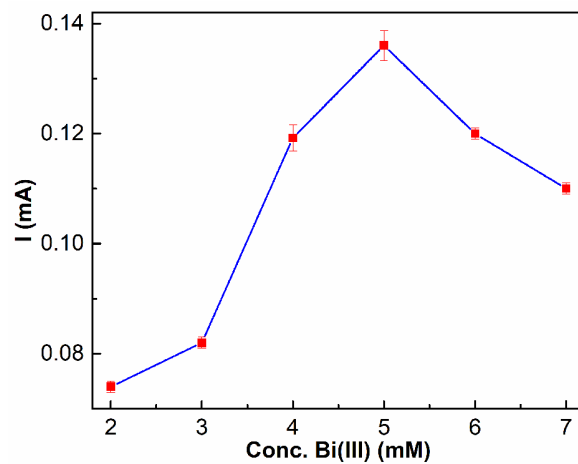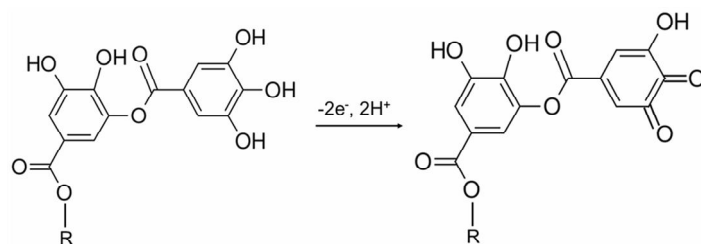

Fig. 7. The influence of bath concentration on cathodic current of 1 µM TA; -450 mV deposition potential and 120 s deposition time.

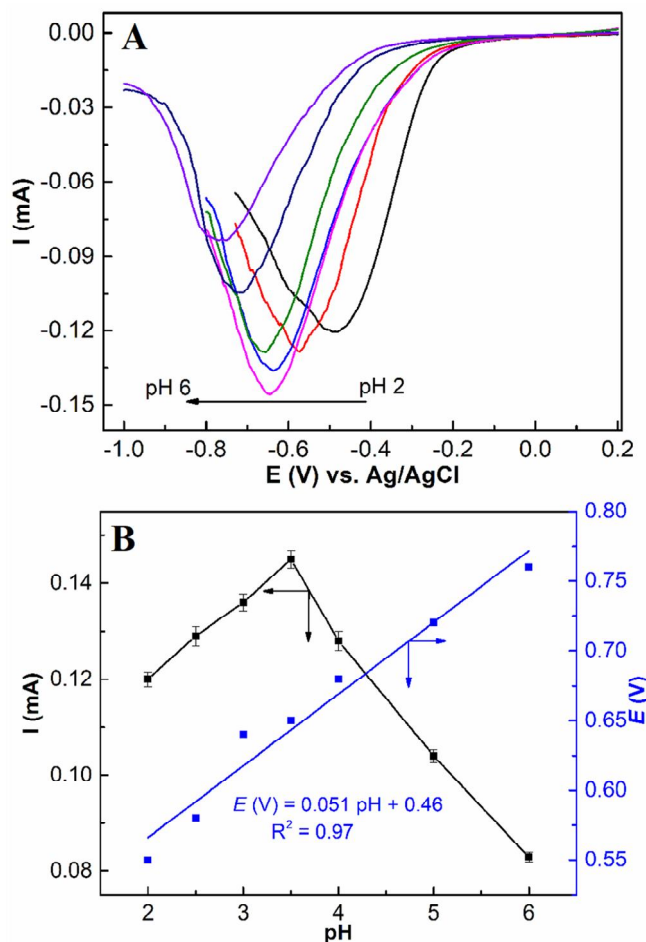*Scheme 1.* Redox mechanisms of a typical polyphenol

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Fig. 8. (A) LSVs of Bi-GCE in 1 μM TA in BR buffer of different pH; (B) The influence of pH of TA on cathodic
current and potential of a solution containing 1 μM TA; 5 mM Bi(III) bath, 120 s deposition time and
-450 mV deposition potential.

the mechanism of TA redox process was proposed in
Scheme 1.

The Study of the Scan Rate Effect

The effect of scan rate was investigated by changing
scan rates from 10 to 300 mV s^{-1} using 1 μM TA in BR
buffer (pH 3.6) at Bi-GCE, and the results are shown in
Fig. 9. It is observed that peak currents are linearly
correlated to the square root of scan rates with regression
equation of y = -0.0103 x + 0.0016 and the square of the
correlation coefficient is about 0.998, implying that the
electrochemical process is a diffusion controlled process.

Calibration Curve

The Bi-GCE presented a definite peak with consistent
peak current values for repetitive determinations. The result
revealed linear relationship in ranges between 0.05-200 μM
(Fig. 10). The obtained linear regression equation for TA
was i(mA) = 0.00296 C (μM) + 0.11 (R^2 = 0.989) (Fig. 10
Inset). The LOD was found to be 0.035 μM (3σ/m), where σ
is the standard deviation of the blank and m is the slope of
the calibration curve.

The performance of this electrode was compared with
other electrodes and the LODs are listed in Table 1. It
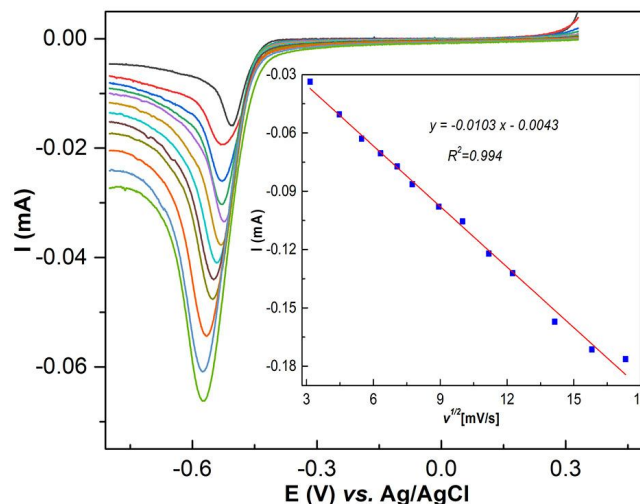can be seen from the table that the demonstrated method

Fig. 9. LSVs of 1 μM TA in BR buffer (pH 3.6) at different scan rate ranging from 10 to 300 mV s^{-1}. (Inset: the corresponding plot of peak current *vs.* square root scan rate).
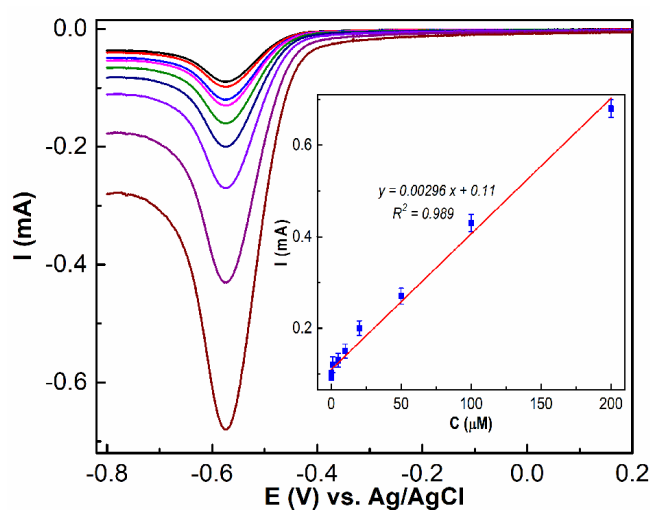

Fig. 10. LSVs of varying concentration of TA (Inset: the corresponding calibration curve from 0.05 μM to 200 μM).

displays an acceptable performance compared to the other methods reported.

Reproducibility and Stability Study of Bi-GCE

To examine the reproducibility of Bi-GCE, three GCEs were coated with Bi film under the same modification conditions. Then, 1 μM TA was measured in triplicate with each Bi-GCE to evaluate the reproducibility, and 4.86% relative standard deviation was obtained for the Bi-GCE. On the other hand, the Bi-GCE showed a low stability upon storage for long time. The cathodic current response of the Bi-GCE declined when TA was measured on the same electrode on successive days. 24% of the reduction current response had declined on the second day of the experiment.

Table 1. Comparison of the LOD of Bi-GCE and Detection Limit with other Reported Electrodes

Electrode	Method	Linear range (µM)	LOD (µM)	Ref.
Multi wall carbon nanotubes modified GCE	LSV	0.4-200	0.1	[48]
Single wall carbon nanotubes modified GCE	DPV	0.05-1	0.008	[49]
Porous pseudo-carbon paste electrode	ASV	0.02-1	0.01	[50]
Silica gel modified carbon paste electrode	ASDPV	0.001-1	0.0003	[51]
Pencil Graphite Electrode	ASDPV	0.005- 0.5	0.0015	[52]
Bi-GCE	LSV	0.05-200	0.035	This work

Table 2. Study the Effect of Interferences

Interferences	Change in reduction currents (%)
Ascorbic acid	-2.91
Glucose	2.78
Uric acid	1.53
VB$_2$	2.02

Table 3. Determination Results of TA in Real Samples

TA added (µM)	TA found (µM)	RSD (%)	Recovery (%)
	0.96	2.84	
1.00	1.88	3.25	95.00
5.00	6.31	3.71	105.90
10.00	10.54	2.53	96.17

After one week, the reduction current response declined further by 62%. This could be due to the formation of a non-electroactive complex between Bi and TA. Therefore, it is suggested that a fresh Bi-GCE should be prepared before the experiment.

Interference Study

In order to show the applicability of the developed method in selectivity of Bi-GCE for the determination of TA, the effect of possible interfering substances was studied under the optimum conditions. Different interfering species were added into the solution containing 1 μM of TA. 0.1 mM ascorbic acid (AA), uric acid (UA), glucose (GL), and vitamin B$_2$ (VB$_2$) had a negligible interference on the determination of 1 μM TA; the result is shown in Table 2.

Real Sample Analysis

For real sample study, 1 ml of tea extracts were spiked with three different concentrations of TA (1.00, 5.00 and 10.00 μM) to each sample. As shown in Table 3, the corresponding percent recovery for the determination of TA in tea sample extracts was in the range of 95.00-105.90% indicating better selectivity for the determination of TA from real sample matrices using Bi-GCE.

CONCLUSIONS

In this work, the electrochemical detection of TA was studied at the Bi-GCE for the first time. The method has shown that the cathodic peak current   is directly related to the concentration of TA in a range from 0.05-200 μM TA with LOD of about 0.035 μM. Compared to the other methods, it has shown the better electroanalytical characteristics such as high sensitivity, lower detection limit and practical applicability. After modification of GCE with bismuth film, the reduction peak current of TA enhanced as compared to the bare GCE. Finally, the proposed method was successfully applied for the detection of TA in tea samples.

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