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Talanta 65 (2005) 511–517
Talanta
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Detection of catechin based on its electrochemical autoxidation

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Received 23 May 2004; received in revised form 7 July 2004; accepted 8 July 2004
Available online 28 August 2004

Abstract

Catechins are strong autoxidant to produce steady intermediate in alkali solution. In present work, we, for the first time, developed a electrochemical method based on ruthenium tris (2, 2′) bipyridyl ($Ru(bpy)_3^{3+}$) modified boron-doped diamond (BDD) electrode to investigate the electrochemical reduction of catechin autoxidation intermediate with its existence verified by the electron spin resonance (ESR). The reduction peak potential was observed at −855.5 mV, not appearing either in acid solution (pH 2) containing catechins or in oxidized catechin solution (pH 12). Moreover, the effects of pH, ascorbic acid, Cu^{2+}, Fe^{2+} and autoxidation time are investigated, demonstrating the reduction peak being really the reduction of catechin autoxidation intermediate. It is found that the peak current is proportional to scan rate, indicative of a surface confined reduction process. Sensitive amperometric response was obtained covering linear range from 0.3268 μM to 0.1591 mM. The determination of catechin in commercial preparations using this method shows satisfactory results comparable with those of the traditional methods.

Keywords: Catechin; Autoxidation; Intermediate; Electrochemical reduction

1. Introduction

Catechins with polyphenol structure [1,2] in vegetables, fruits and teas can prevent degenerative diseases including cancers and heart diseases through antioxidative action of several protein functions, mainly due to their antioxidant activities and abilities to scavenge free radicals, such as $O_2^{\bullet -}$, $OH^{\bullet}$ etc [3–7]. On the other hand, catechins are susceptible to autoxidation to generate free radical intermediate, and active oxygen species, such as $O_2^{\bullet -}$ and hydrogen peroxide (H_2O_2) [8,9] to induce DNA damage and diseases. In the autoxidizing process, cupric ion (Cu^{2+}) that is subject to be reduced, can improve the rate of autoxidation and generation of free radical intermediate [10,11]. There is growing evidence that ascorbic acid has a pro-oxidant role for catechins because it can scavenge free radicals [12,13]. On the contrary, borate is proposed to inhibit the autoxidation of catechin [9]. However, there are rare reports concerning the characteristics of the electrochemical reduction of catechin autoxidation intermediate and the use of this electrochemical reduction to detect catechins.

The traditional methods for detecting catechins include high-performance liquid chromatography (HPLC) combined with chemiluminescence [14], electrochemical [15,16], UV and fluorimetric [17] detection. The capillay electrophoresis with electrochemical detection was also developed to determine catechins [18]. Romani et al. [19] compared HPLC/DAD analysis with screen-printed electrodes and biosensors for polyphenol determination. Most accurate data were obtained by HPLC/DAD method. The electrode was considered as a quick screening method, and the use of biosensors needs further improvement of the performance.

In present work, we developed ruthenium tris (2, 2′) bipyridyl ($Ru(bpy)_3^{3+}$) modified boron-doped diamond (BDD) electrode to investigate the characteristics of the electrochemical reduction of catechin autoxidation intermediate using the electrochemistry to detect total catechins. An elec-

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doi:10.1016/j.talanta.2004.07.045

tron spin resonance (ESR) experiment was carried out to verify the existence and steadiness of the intermediate. The effects of ascorbic acid, Cu^{2+}, Fe^{2+}, H^+ and autoxidation time were investigated to prove the reduction peak being really the electrochemical reduction of catechin autoxidation intermediate. Satisfactory linear range of catechin assay using chronoamperometry (CA) and commercial preparation detection were obtained.

2. Experimental

2.1. Apparatus and materials

All cyclic voltammograms and chronoamperometry data were acquired using a computer-based potentiostate/galvanostate (model 283) (EG & GP Princeton Applied Research, Princeton, NJ, USA). ESR spectra were recorded by EMX 10/12 EPR Spectrometer (USA). The three-electrode system consists of a $Ru(bpy)_3^{3+}$ boron-doped diamond electrode, a saturated calomel reference electrode and a platinum wire auxiliary electrode.

The boron-doped diamond was kindly provided by College of Materials Science and Engineering of Hunan University. $Ru(bpy)_3^{3+}$ was purchased from Sigma. Catechin was offered by Hunan Agricultural University. The catechin solutions were freshly prepared immediately before use. Other chemicals are of analytical reagent grade. Doubly distilled water was used throughout. The supporting electrolyte was 0.1 mol L^{-1} $NaNO_3$ adjusted to different values of pH with H_2SO_4 and NaOH.

2.2. Preparation of oxidized catechin solution

Catechin solution of 1 mM in 0.1 M $NaNO_3$ of pH 12 was oxidized by $K_3Fe(CN)_6$. During the process of oxidation, the temperature was kept at 0 °C with an oxidation time of 30 min [20]. Then oxidized catechin solution of 1 mM concentration was prepared.

2.3. Fabrication of $Ru(bpy)_3^{3+}$-modified oxidized BDD and glass carbon electrode

The BDD and glass carbon electrodes were subjected to electrolytic oxidation in a 1 mol L^{-1} H_2SO_4 aqueous solution at 3.0 V versus SCE with a platinum wire auxiliary electrode for 30 min to introduce hydroxyl groups to its surface [21]. The surface was then electrochemically modified with ruthenium tris (2, 2′) bipyridyl, for this purpose the oxidized BDD and glass carbon electrodes were scanned 20 circles using cyclic voltammetry (CV) in 1 mmol L^{-1} $Ru(bpy)_3^{3+}$ with potential range from −1000 to 1000 mV and scan rate of 100 mV s^{-1}. Thus, $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode and $Ru(bpy)_3^{3+}$-modified oxidized glass carbon electrode were prepared.

2.4. Procedure

The chronoamperometry experiments of catechin with $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode as working electrode were carried at an applied potential −750 mV and a time period of is 180 s. The solutions of catechin were kept in 0.1 M $NaNO_3$ with pH 12.

3. Results and discussion

3.1. ESR study of catechin autoxidation intermediate

The ESR spectrum of catechin autoxidation intermediate in 0.1 M $NaNO_3$ of pH 12 is shown in Fig. 1a. A large signal corresponding to free radical is observed which is similar to that reported for the free radical intermediate from catechin autoxidation in 1 M NaOH [21]. After the catechin solution being placed in atmosphere for 30 min, the change of the signal is hardly observable as shown in Fig. 1b. No spectral signals were observable in 0.1 M $NaNO_3$ of pH 2 containing 1 mM catechin (Fig. 1c) or catechin solution after oxidation (Fig. 1d). The results indicate that catechin autoxidation intermediate is a free radical intermediate, being only generated during catechin autoxidation in 0.1 M $NaNO_3$ of pH 12 and exists steadily.

3.2. Electrochemical characteristics of catechin autoxidation intermediate on $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode

Fig. 2a shows the cyclic voltammograms obtained with the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode in 0.1 mol L^{-1} $NaNO_3$ (pH 12) containing 1 mmol L^{-1} catechin. A sharp reduction peak is apparent at −855.5 mV with

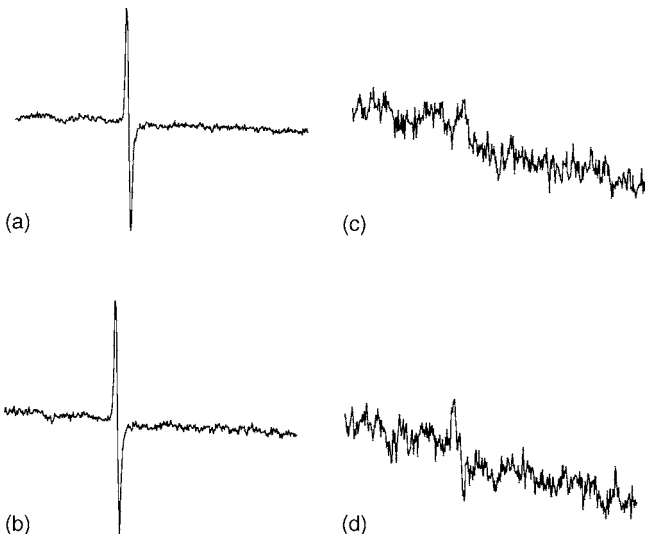


Fig. 1. ESR spectra of 1 mM catechin in 0.1 M $NaNO_3$ of pH 12 (a) immediately after preparation and (b) after standing for 30 min, (c) in 0.1 M $NaNO_3$ of pH 2 and (d) in oxidized I mM catechin.

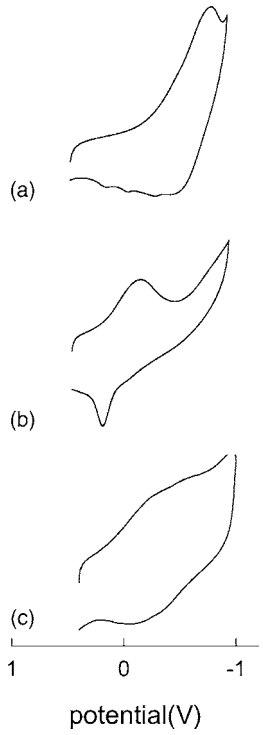


Fig. 2. Cyclic voltammograms obtained with the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode (a) in 0.1 M $NaNO_3$ of pH 12 and (b) pH 2 containing 1mM catechin and (c) in 0.1 M $NaNO_3$ of pH 12 containing 1 mM oxidized catechin. Scan rate: 50 mV s^{-1}, potential range: 0.4 to −1.0 V.

no apparent oxidation peak. This reduction peak has not been observed on the cyclic voltammogram with the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode in 0.1 mol L^{-1} $NaNO_3$ (pH 2) containing 1 mmol L^{-1} catechin, as shown in Fig. 2b, while a couple of reduction and oxidation peaks at −209.8 and 127.0 mV, respectively, appears. The results are consistent with the previous literature [22] which reported that catechin can oxidize at 120–220 mV in acid and neutral solutions. No sharp reduction and oxidation peaks were observed on the cyclic voltammogram obtained in oxidized catechin solution as shown in Fig. 2c. A small reduction peak is at around −200 mV, similar to the reduction peak on Fig. 2b. Combined with the results of Fig. 1, the reduction peak may be from the reduction of free radical intermediate.

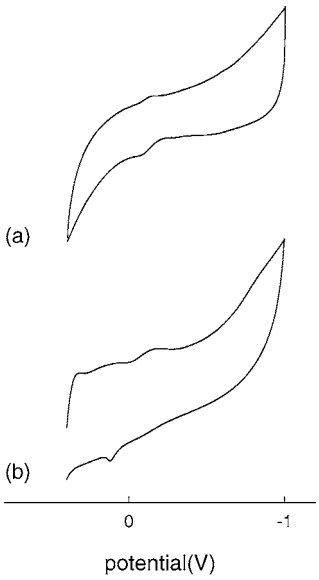


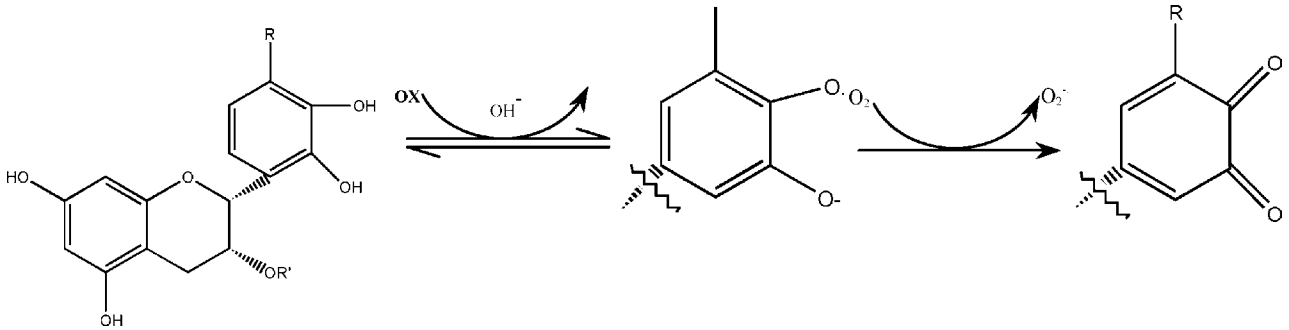
Fig. 3. Cyclic voltammograms obtained with the $Ru(bpy)_3^{3+}$: (a) modified oxidized GC electrode and (b) the oxidized BDD electrode in 0.1 M $NaNO_3$ of pH 12 containing 1mM catechin. Scan rate: 50 mV s^{-1}, potential range: 0.4 to −1.0 V.

Fig. 3 shows the cyclic voltammograms obtained with the $Ru(bpy)_3^{3+}$-modified oxidized glass carbon electrode(a) and the oxidized BDD electrode (b) in 0.1 mol L^{-1} $NaNO_3$ (pH 12) containing 1 mmol L^{-1} catechin. There is no sharp reduction peak on curve a, but a couple of redox peaks can be observed, which may be attributed to reduction and oxidation of catechin. On the oxidized BDD electrode, small redox peak potential is similar with that in Fig. 2b. The results show that only $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode can figure out the reduction of catechin autoxidation intermediate.

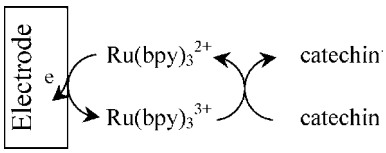
3.3. Mechanism of free radical intermediate being monitored on $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode

It has been reported [9] that the process of catechin autoxidation can be expressed as follows: Scheme 1.

At room temperature, the rate of autoxidation is very slow [8]. In our experiment, $Ru(bpy)_3^{3+}$ film on the BDD electrode can accelerate formation of the intermedi-



Scheme 1.



Scheme 2.

ate, due to $Ru(bpy)_3^{3+}$ working as a one-electron oxidant [23](Figs. 2a and 3b has proved it). The probable process is as follows: Scheme 2.

Fig. 1a and b demonstrate that free radical intermediate can exist steadily in pH 12 solution. Carbon sp^3 and boron possess the electron-deficient structure, which is subject to combine with free radical intermediates to be accumulated on BDD electrode surface. The results of Fig. 2a and 3a have proved the speculation. Owing to these kinds of actions, the free radical intermediate can be reduced electrochemically and monitored on $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode.

3.4. pH dependence of the autoxidation

In 0.1 M $NaNO_3$ solution containing 1 mM catechin, the effect of pH on the reduction peak current and potential E_p was examined in pH range of 9.0–12 as shown in Fig. 4. The peak current increases parallel with the increasing pH, which means the electrochemical reduction rate increases with pH. Additionally, the peak potential of reduction peak shifts in negative direction with increasing pH. The relationship of peak potential (mV) with pH is $-E_p = 354.4 + 41.05\text{pH}$. This indicates H^+ joins in the catechin autoxidation intermediate electrochemical reaction after the intermediate capturing one electron [24]. The process may be as follows: Scheme 3.

3.5. Effect of autoxidation time

The relationship between peak current and autoxidant time from 0 to 210 min was monitored as shown in Fig. 5. The peak

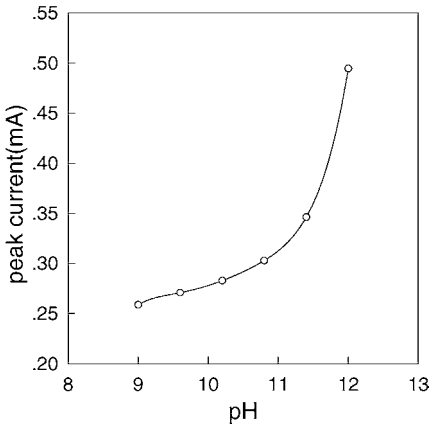
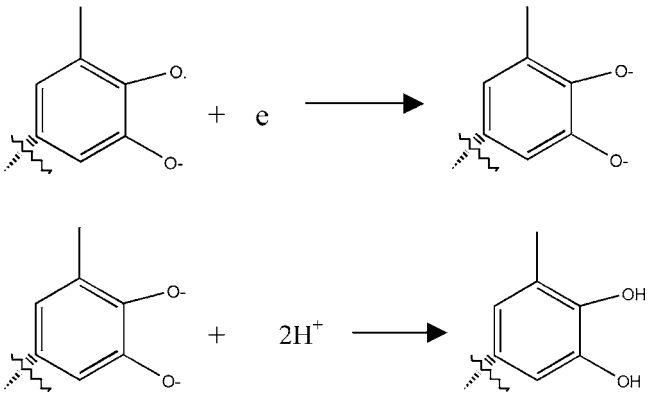


Fig. 4. Relationship between pH and electrochemical reduction peak current on the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode studied by cyclic voltammetry in 0.1 $NaNO_3$ containing 1 mM catechin.



Scheme 3.

current increases dramatically during 60 min. From 60 to 120 min, the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode has maximum sensitivity. While autoxidant time is more than 120 min, the peak current decreases. At the beginning of autoxidation, the rate of autoxidation reaction is low because of lack of O_2 and $OH^\bullet$ [8]. With the increase of time, the amount of $OH^\bullet$ and O_2 dissolved in solution increases and the rate is fast and the amount of the intermediate increases, too. At certain time, the amount of the intermediate reaches the maximum and keeps the value in a time range and a platform appears on Fig. 5. With the decrease of catechin in solution, the peak current decreases with the amount of the intermediate produced decreasing.

3.6. Effect of ascorbic acid

Ascorbic acid concentration affects the reduction peak current. The peak current decreases gradually with the increase of ascorbic acid concentration. On the other hand, an oxidation peak appears and the peak current increases with the increase of ascorbic acid, which may be due to the oxidation of ascorbic acid. It is known that ascorbic acid is a quenching agent of free radical. It makes the free radical in-

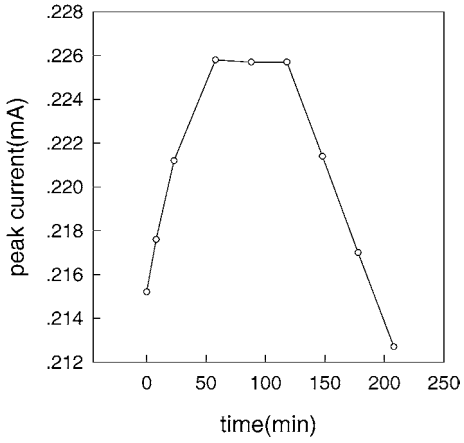


Fig. 5. Dependence of peak current on autoxidation time at the electrode in pH 12, 0.1 $NaNO_3$ containing 1 mM catechin.

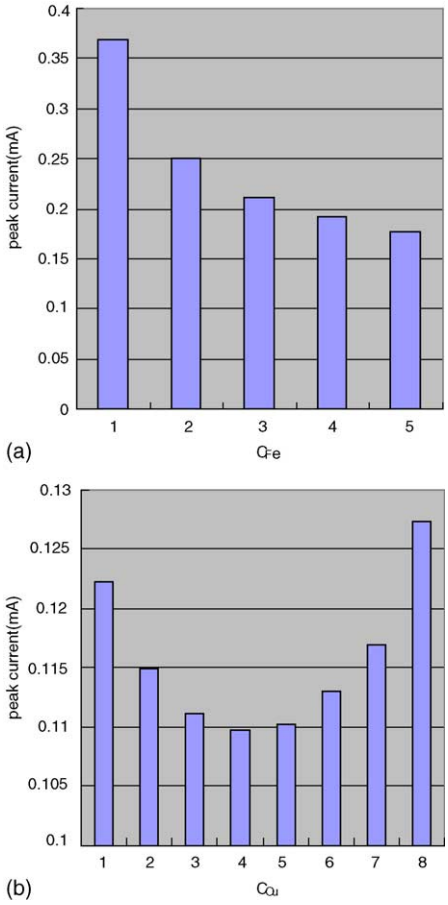


Fig. 6. (a) Effect of Fe^{2+} on the reduction peak current (from column 1–5: 0.5, 1, 1.5, 2, and 2.5 mM). (b) Effect of Cu^{2+} on the reduction peak current (from column 1–8: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM).

termediate being oxidized before reaching the surface of the electrode. The result provides a evidence that the reduction peak comes really from the reduction of free radical intermediate on the $Ru(bpy)_3{}^{3+}$-modified oxidized BDD electrode.

3.7. Effect of Fe^{2+} and Cu^{2+}

It has been reported that metal ions have effects on hydrogen transfer from oxidant chemical to free radical using UV–vis spectroscopy [25]. Therefore, we investigated the effect of Fe^{2+} on the reduction peak current as shown in Fig. 6a.

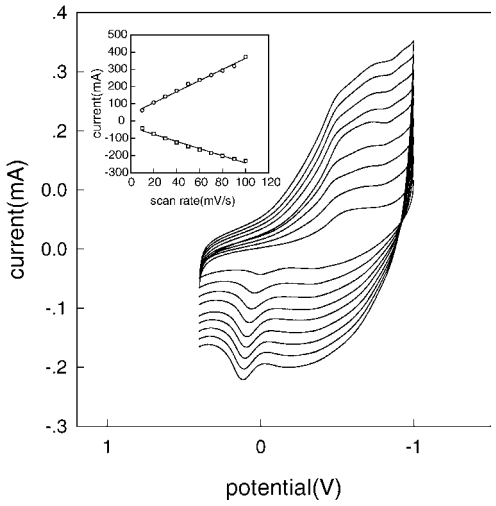


Fig. 7. Cyclic voltammograms obtained with the $Ru(bpy)_3{}^{3+}$-modified oxidized BDD electrode in 0.1 M $NaNO_3$ of pH 12 containing 1 mM catechin at various scan rate. (from inner to outer curve: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mV s^{-1}).

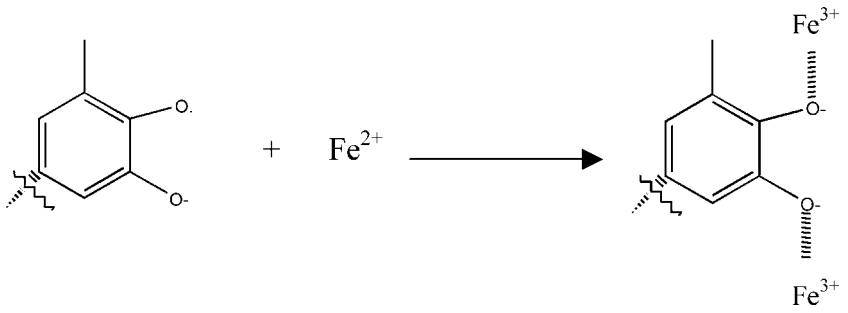
One notices that the peak current decreases with the increase of Fe^{2+}. The result may be attributed to the intermediate being reduced by Fe^{2+} in solution and the strong binding of Fe^{3+} to reduction product. Then the amount of catechin autoxidation intermediate decreases on the surface of electrode. The process is possible as follows: Scheme 4.

Cu^{2+} acts as a one-electron oxidant in place of O_2 [10,11]. The possible process is following: Scheme 5.

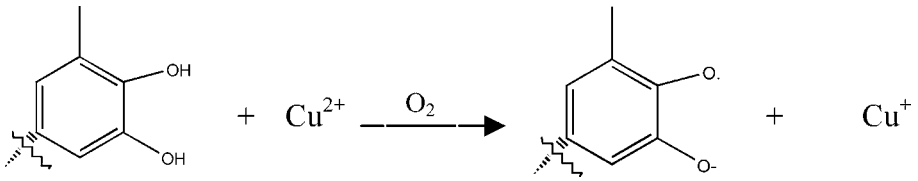
Cu^{2+} can induce catechin to produce the free radical intermediate. Fig. 6b shows the relationship between Cu^{2+} concentration and the reduction peak current. The peak current decreases parallel with the increase of Cu^{2+} in low Cu^{2+} concentration. When the Cu^{2+} concentration is greater than 1.5 mmol L^{-1}, the reduction peak current increases with the increase of Cu^{2+} concentration. There is a "lag period" in low Cu^{2+} concentration, which may be dependent on the lack of molecular oxygen initially present.

3.8. Effect of scan rate

The influence of potential scan rate on the oxidation and reduction peak current has been investigated by cyclic voltammetry in the range of 10–100 mV s^{-1} as shown in Fig. 7. It



Scheme 4.



Scheme 5.

can be observed that the redox peak current is proportional to the scan rate, indicative of a surface confined redox process. Other phenomena are found that the oxidation peak of catechin appears at high scan rate and the reduction peak becomes broader with the increase of scan rate. The results indicate that low scan rate is suitable for the reduction of catechin autoxidation intermediate.

3.9. Effect of catechin concentration on the cyclic voltammetry response

The reduction peak current was investigated at the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode using different concentrations from 0.01 to 1 mM, as shown in Fig. 8. With the concentrations increasing, the peak potential appears to decrease slightly. The peak current is inversely proportional to catechin concentration. Linear regression of peak current (μA) versus concentration (mM) profiles shows reasonable linearity from 0.01 to 0.8 mM (slope = $-0.02249\,\mu A\,mM^{-1}$, linear equation: $I = -0.02249 \log C - 0.04303$). The possible reason is that it is beneficial for the second step in Scheme 1 in high catechin concentration, but not so for the rate of second step in Scheme 3.

3.10. Application of $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode

Fig. 9 shows the effect of applied potential on $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode CA response. With the increasing potential from −500 to −700 mV, the sensitivity of the modified electrode increased significantly. The sensitivity obtained at potential lower than −700 mV actually leveled off. A potential of −750 mV (versus SCE), which is close to the reduction peak potential, was selected as the applied potential for amperometric measurements.

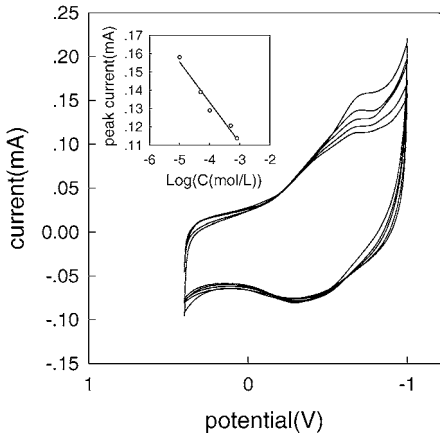


Fig. 8. Cyclic voltammograms obtained with the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode in 0.1 M $NaNO_3$ of pH 12 containing different amount of catechin.

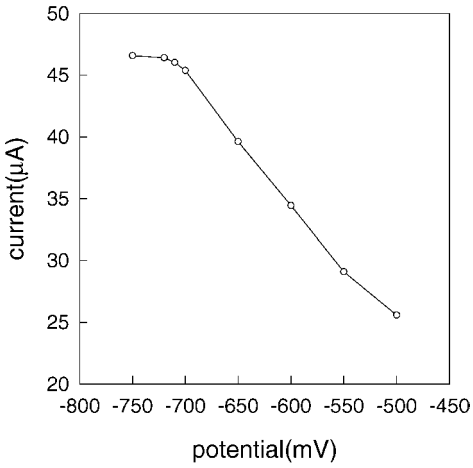


Fig. 9. Dependence of steady-state current on applied potential at the electrode in 0.1 M $NaNO_3$ of pH 12 containing 1 mM catechin.

The calibration curve for catechin using CA with an applied potential of −750 mV. A linear relationship between the peak current and the logarithm of catechin concentration is obtained covering the range from 0.3286 μM to 0.1591 mM, the linear regression equation being $I = -33.96 \log C - 204.18$ with a correlation coefficient of 0.9864. The detection limit is $1.21 \times 10^{-7}\,mol\,L^{-1}$.

The proposed method was used for the determination of catechin in commercial preparations, which purchased from Yalong Company in China. The results were compared with those the traditional HPLC method (Table 1).

Table 1
Determination of catechin in commercial preparations

The electrode method (%)	Mean (%)	RSD (%)	The HPLC method (%)
70.37 61.20 65.86 70.71	67.04	3.9	67.73

4. Conclusion

We have successfully investigated the characteristics of electrochemical reduction of the intermediate produced in the process of catechin autoxidation using $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode. $Ru(bpy)_3^{3+}$ film on the oxidized BDD electrode accelerates the rate of catechin autoxidation to produce the intermediate on electrode surface. Carbon sp^3 and boron in BDD electrode possess the electron-deficient structure that is subject to combine with catechin autoxidation intermediate. On the basis of interactions between the $Ru(bpy)_3^{3+}$-modified oxidized BDD electrode and catechin autoxidation intermediate, the reduction characteristics could be monitored. The reduction process is a surface confined one, which further confirm the electrode surface to play a critical role for the electrochemical reduction of the intermediate.

The peak potential and current is dependent on pH. H^+ joins in the intermediate electrochemical reduction after capturing one electron (Scheme 3). Fe^{2+} causes the electron reduction of the intermediate in solution (Scheme 4) and hinders its electrochemical reduction on the surface of electrode. Ascorbic acid cuases the decrease of reduction peak current due to the oxidation of the intermediate. The existence of Cu^{2+} is beneficial for the increase of reduction peak current. These effects further show that the process of electrochemical reduction of intermediate followed Scheme 3.

Based on the characteristics of the electrochemical reduction, satisfactory results of catechin determination were obtained. The peak current is inversely proportional to catechin concentration. The possible reason is that it is beneficial for the second step in Scheme 1 in high catechin concentration and inverse side for the rate of second step of in Scheme 3. The results of catechin sample assay in commercial preparations were accurate compared with those of HPLC.

References

[1] R. Pulido, L. Bravo, F. Saura-Calixto, J. Agric. Food Chem. 48 (2000) 3396–3402.
[2] H. Sakakibara, Y. Honda, S. Nakagawa, J. Agric. Food Chem. 51 (2003) 571–581.
[3] N. Zhu, T.C. Huang, Y. Yu, E.J. Lavoie, C.S. Yang, C.T. Ho, J. Agric. Food Chem. 48 (2000) 979–981.
[4] K. Fukuhara, I. Nakanisi, H. Kansui, E. Sugiyama, M. Kimura, et al., J. Am. Chem. Soc. 124 (2002) 5952–5953.
[5] D. Rosch, M. Bergmann, D. Knorr, L.W. Kroh, J. Agric. Food Chem. 51 (2003) 4233–4239.
[6] M.L. Andersen, H. Outtrup, L.H. Skibsted, J. Agric. Food Chem. 48 (2000) 3106–3111.
[7] S. Sang, X. Cheng, R.E. Stark, R.T. Rosen, et al., Bioorg. Med. Chem. 10 (2002) 2233–2237.
[8] Y. Guo, G. Zou, Amino acids Biotic Res. 23 (2) (2001) 10–12.
[9] M. Mochizuki, S. Yamazaki, K. Kano, T. Ikeda, Biochimica Biophysica Acta 1569 (2002) 24–35.
[10] M. Hosny, J.P.N. Rosazza, J. Agric. Food Chem. 50 (2002) 5539–5545.
[11] A.C. Clark, P.D. Prenzler, G.R. Scollary, J. Agric. Food Chem. 51 (2003) 6204–6210.
[12] M.P. Bradshaw, V. Cheynier, G.R. Scollary, P.D. Prenzler, J. Agric. Food Chem. 51 (2003) 4126–4132.
[13] M.P. Bradshaw, P.D. Prenzler, G.R. Scollary, J. Agric. Food Chem. 49 (2001) 934–939.
[14] K. Nakagawa, T. Miyazawa, Anal. Biochem. 248 (1997) 41–49.
[15] B. Yang, K. Arai, F. Kusu, Anal. Biochem. 283 (2000) 77–82.
[16] M.J. Lee, S. Prabhu, X. Meng, C. Li, C.S. Yang, Anal. Biochem. 279 (2000) 164–169.
[17] M.A. Rodriguez-Delgado, S. Malovana, J.P. Perez, T. Borges, J. Chrom. A 912 (2001) 249–257.
[18] Y. Peng, Q. Chu, F. Liu, J. Ye, J. Agric. Food Chem. 52 (2004) 153–156.
[19] A. Romani, M. Minunni, N. Mulinacci, P. Pinelli, F.F. Vincieri, J. Agric. Food Chem. 48 (2000) 1197–1203.
[20] D. Li, C. Wan, X. Xiao, Chaye Tongbao 22 (2) (2000) 17–18.
[21] W. Li, Q. Guo, L. Zhao, R. Shen, F. Li, W. Hou, Chin. J. Magn. Reson. 15 (6) (1998) 507–512.
[22] V. Roginsky, T. Barsukova, C.F. Hsu, P.A. Kilmartin, J. Agric. Food Chem. 51 (2003) 5798–5802.
[23] W. Miao, J. Choi, A.J. Bard, J. Am. Chem. Soc. 124 (2002) 14478–14485.
[24] J. Song, H. Fu, W. Guo, Anal. Lett. 33 (11) (2000) 2259–2269.
[25] I. Nakanishi, K. Miyaki, T. Shimada, K. Ohkubo, S. Urano, N. Ikota, T. Ozawa, S. Fukuzumi, K. Fukuhara, J. Phy. Chem. A 106 (2002) 11123–11126.