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Catechin electrochemical oxidation mechanisms

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Abstract

The oxidation of flavonoids is of great interest because of their action as antioxidants with the ability to scavenge radicals by electron transfer processes. The electrochemical oxidation of the flavonoid (+)-catechin, was investigated, over a wide range of conditions, using cyclic, differential and square wave voltammetry. The oxidation mechanism proceeds in sequential steps, related with the catechol and resorcinol groups and the oxidation is pH dependent. The oxidation of the catechol 3′,4′-dihydroxyl electron-donating groups occurs first, at very low positive potentials, and is a reversible reaction. The hydroxyl groups of the resorcinol moiety oxidised afterwards were shown to undergo an irreversible oxidation reaction. (+)-Catechin also adsorbs strongly on the electrode surface and the final oxidation product is not electroactive and blocks the electrode surface.

Keywords: Catechin; Electrochemistry; Oxidation; Radical scavenging activity; Flavonoids; Free radicals

1. Introduction

Polyphenols, flavonoids, and tannins are widely distributed in plants and are known to exhibit higher antioxidant activities [1–3]. Also, polyphenols in the human diet may exert a beneficial health effect protecting against some diseases, including coronary heart disease and some cancers [4]. Antioxidants are of great interest because of their involvement in important biological and industrial processes. Flavanols found in green tea, are among the most antioxidant active compounds researched thus far [5].

It has been shown that the antioxidant activity of flavonoids is due to the aromatic OH groups [6]. The reactive structural groups for the flavonoids are: (1) the pyrogallol group; (2) the catechol group; (3) the 2,3-double bond in conjugation with a 4-oxo group and a 3-hydroxyl group; (4) additional resonance-effective substituents [7].

The chemical properties of polyphenols in terms of the availability of the phenolic hydrogens as hydrogen-donating radical scavengers suggest they will have antioxidant activity. To be defined as an antioxidant a polyphenol must satisfy two basic conditions. First, when present in low concentration relative to the substrate to be oxidised it delays, retards or prevents autoxidation or free radical-mediated oxidation [8]. Secondly, the resulting radical formed after scavenging must be stable—through intramolecular hydrogen bonding [5,9].

Phenolic compounds are members of the groups of hormones, vitamins, and food antioxidants. The mechanism of their action as antioxidants seems to involve the ability of phenols to scavenge radicals by electron transfer process by which the phenol is converted into a phenoxyl radical. The phenolates were described as being more easily oxidised than phenols [10]. Due to the rapid formation of phenolate ions at the electrode surface, its oxidation is favoured for pH values well below their pK_a. A detailed pH dependent mechanism of the oxidation of the phenolate anions etoposide and tenoposide was also reported [10]. However, as long as the formation of the phenolate is fast enough, it is the phenolate which is oxidised first.

Many studies focused on the protective effects of flavan-3-ols against lipid peroxidation and low-density lipoproteins (LDL) oxidation as well as on their antiproliferative and anticarcinogenic actions [11]. These protective effects have been mainly attributed to the antioxidative activities of flavan-3-ols and their activity to inhibit enzymes involved in the production of reactive oxygen species (ROS). There is much discussion and contradiction regarding the structure of the phenoxyl radicals, the reduction potential

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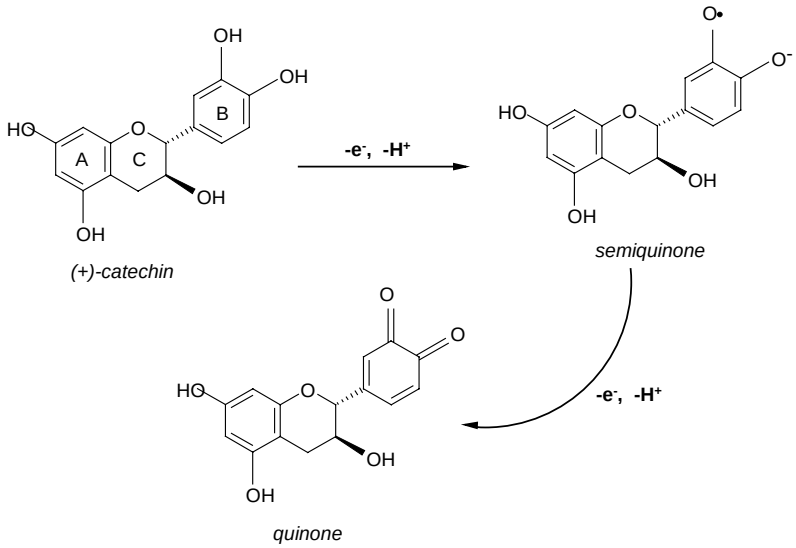

Scheme 1. Mechanism of oxidation of catechin.

and the structure-activity relationship on the antioxidant activity.

The flavonoid (+)-catechin has two different pharmacophores, the catechol group in ring B and the resorcinol group in ring A and it has also the hydroxyl group at position 3 in ring C, see Scheme 1. Most of the problems encountered when describing catechin antioxidative activity are due to the lack of information on the intrinsic reactivity of each ring (A, B) and the lack of reliable thermodynamic constants.

The A and B rings of (+)-catechin are not conjugated and ionisation of the OH groups of one ring system should not appreciably affect ionisation of the OH groups of the other ring. Hence, ionisations of OH groups of ring A are independent and distinguishable from those of ring B [12]. It was concluded by spectroscopic methods that the influence of the A ring on the spectral properties of the radicals from the B ring is negligible in flavonoids where the C ring is completely saturated [13].

Cyclic voltammetry has been used for the evaluation of antioxidant capacity of several polyphenols and their mixtures [14,15]. The redox properties of polyphenols have been utilised as a measure of the antioxidant properties of wines on the basis of the measurement of the oxidation current at a constant potential by HPLC with electrochemical detection [16]. So far, few attempts have been made to pursue a possible correlation between the oxidation potentials of antioxidants and their antioxidant activities.

The phenolic groups of flavonoids can be electrochemically oxidised, and most flavonoids show an oxidation peak [7]. Several antioxidants with two oxidizable moieties were studied by cyclic voltammetry and their free radical scavenging activity against the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also determined [17]. The mechanism of oxidation of catechins using electron spin resonance (ESR) data has been proposed [18], as shown in Scheme 1, for (+)-catechin. Many published reports described the determination and quantification of catechins, using HPLC with ultraviolet [19,20], fluorescence [21], mass spectroscopy [20] and electrochemical [22,23] detection. The reduction potentials of flavonoids depend strongly on the electron-donating properties of the substituents in the B-ring [13].

In this study the electrochemical mechanism of oxidation of (+)-catechin was investigated, for a wide range of solution conditions, using cyclic, differential and square wave voltammetry. Some information on the mechanism of (+)-catechin oxidation obtained from results at different pH may play a crucial role in understanding its antioxidant activity.

2. Experimental

The (+)-catechin (*trans*-3,3′,4′,5,7-pentahydroxyflavane) of 98% purity was from Sigma–Aldrich, Madrid, Spain, and all the other reagents were Merck analytical grade. All solutions were made using deionised water obtained from a Millipore Milli-Q purification system (resistivity $\geq$ 18 MΩ cm). The 10 mM stock solution of (+)-catechin was prepared in 100% ethanol.

All experiments were carried out at room temperature (ca. 22±1 °C) and in the presence of dissolved oxygen. Solutions of buffer supporting electrolyte of ionic strength 0.2 were used in all experiments (Table 1).

The pH measurements were carried out with a CRISON GLP 21 pH-meter.

Electrochemical experiments were done using an Autolab PGSTAT 10 running with GPES (General-Purpose Electrochemical System) version 4.9, software (Eco-Chemie, Utrecht, The Netherlands). Voltammetric curves were recorded at room temperature using a three-electrode system in a small-volume electrochemical cell of capacity

Table 1
Supporting electrolyte solutions

Solution 1	Solution 2	pH
0.2 M KCl	0.2 M HCl	1.1
0.2 M KCl	0.2 M HCl	2.1
0.2 M NaOAc	0.2 M HAcO	3.5
0.2 M NaOAc	0.2 M HAcO	4.5
0.2 M NaOAc	0.2 M HAcO	5.2
0.2 M Na_2HPO_4	0.2 M NaH_2PO_4	6.0
0.2 M Na_2HPO_4	0.2 M NaH_2PO_4	7.0
0.2 M Na_2HPO_4	0.2 M NaH_2PO_4	8.0
0.025 M $Na_2B_4O_7{\cdot}10H_2O$	0.1 M NaOH	8.7
2 M NH_3	2 M NH_4Cl	9.2
2 M NH_3	2 M NH_4Cl	10.0
2 M NH_3	2 M NH_4Cl	11.2
0.2 M KCl	0.2 M NaOH	12.0
0.2 M KCl	0.2 M NaOH	12.5

2 mL (Cypress System Inc., USA). The working electrode was a glassy carbon mini-electrode of 1.5 mm diameter; Ag/AgCl (saturated KCl) was used as a reference electrode and a platinum wire as counter electrode. In this work, all potentials were reported versus Ag/AgCl (sat) electrode. The glassy carbon working electrode was polished with diamond spray (6 and 1 μm). Cyclic voltammograms were performed at scan rates of 25, 50, 100 mV s^{-1}. Differential pulse voltammetry conditions used were pulse amplitude 50 mV, pulse width 70 ms, and scan rate of 5 mV s^{-1}. Square wave voltammetry conditions were frequency 13, 25 and 50 Hz, amplitude 50 mV, and potential increment 2 mV (effective scan rates were 25, 50 and 100 mV s^{-1}, respectively). Voltammetric scans were carried out in the potential range −1.0 to +1.4 V versus Ag/AgCl.

3. Results

The structure of (+)-catechin has functional OH groups attached to ring structures that can be electrochemically oxidised. Electrochemical studies reveal general trends in the electron-donating abilities of flavonoids. It was demonstrated that the catechol B-ring is more easily oxidizable than the resorcinol A-ring, and on the B-ring, the most oxidizable phenolic function is the more basic site [11]. The pK_a values were assigned to ring A and ring B following reference [12].

The electronic transfer occurs selectively at the ring with the lower redox potential, i.e. the catechol B-ring. Theoretical calculations of the stability of the various catechin radicals confirmed these trends: the 4′-phenoxyl radical was the most stable radical, and the radicals were ordered with regard to their values ($E_{PhO^{\bullet}} - E_{PhO^{-}}$) characterising the electron affinity in the following sequence: 4′-OH, 3′-OH, 7-OH, 5-OH [11].

Cyclic voltammetry of (+)-catechin at pH 8.0 showed two oxidation peaks associated with oxidation centres present in the molecule: a reversible peak 1 at +0.16 V, confirmed in the second scan by inverting the potential scan just before peak 2, and an irreversible peak 2 at +0.58 V (Fig. 1). The reduction peak at +0.11 V corresponds to the reduction of the oxidation products formed in peak 1. The reversibility of peak 1 was detected over the whole pH range studied although the current was higher and the separation between oxidation and reduction peaks was smaller at pH 7.0 (Fig. 2). The oxidation products formed in peak 1 were reversibly reduced and were also oxidised at higher potentials. The oxidation which occurs in peak 2 was always irreversible for all pHs studied. Moreover, (+)-catechin adsorbs strongly on the electrode surface and the final oxidation product blocks the electrode surface, as demonstrated by the rapid decrease of the (+)-catechin oxidation peak 1 on repeated cycling.

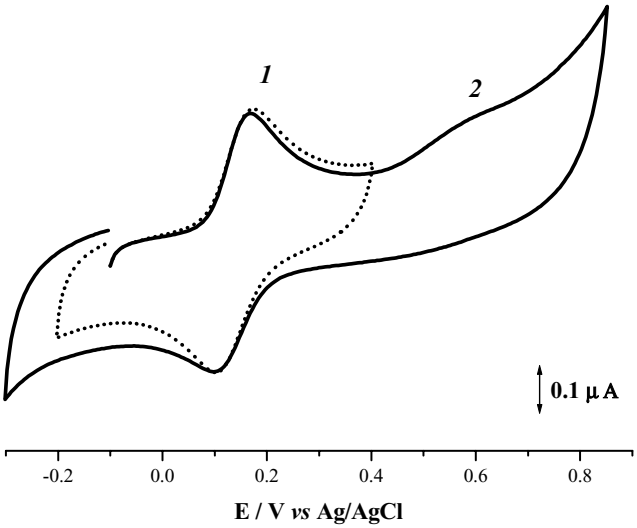

Fig. 1. Cyclic voltammograms of 1 mM (+)-catechin in pH 8.0 phosphate buffer. Scan rate 50 mV s^{-1}.

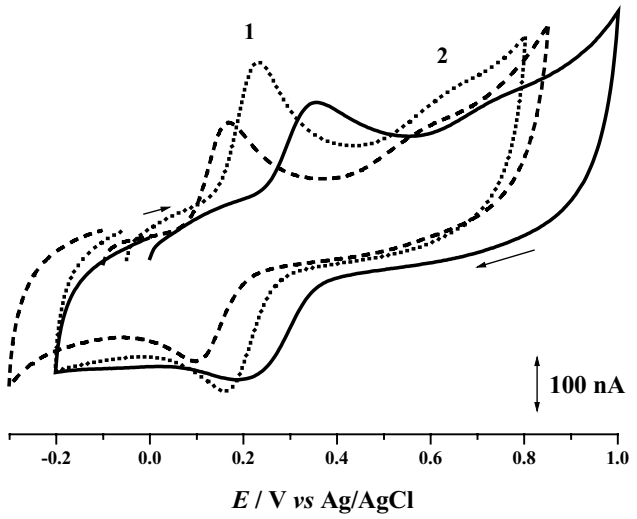

Fig. 2. Cyclic voltammograms of 1 mM (+)-catechin: (—) pH 5.2 acetate buffer; (. . .) pH 7.0 phosphate buffer; (- - -) pH 8.0 phosphate buffer. Scan rate 50 mV s^{-1}.

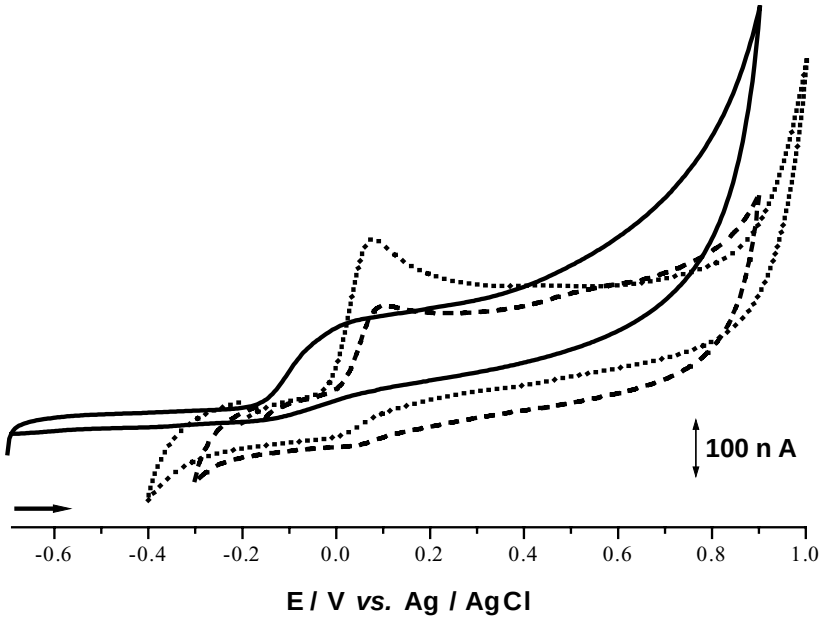

Fig. 3. Cyclic voltammograms of 1 mM (+)-catechin: (—) pH 9.7 ammonium chloride buffer; (. . .) pH 10.0 ammonium chloride buffer; (- - -) pH 12.5 NaOH/KCl supporting electrolyte. Scan rate 50 mV s^{-1}.

Some information on the mechanism of polyphenol oxidation was provided by comparing oxidation potentials of peak 1 at different pH values. Fig. 2 shows that an increase of pH is associated with a decrease of the oxidation potential and it was also found that at high pH the reduction peak 1 almost disappeared (Fig. 3). The same was found during the oxidation of luteolin [24]. From this, it was concluded that at high pH *o*-quinones generated in the reaction are unstable. The reaction at higher pH follows a different pathway than at lower pH and the reaction products are easily oxidised, i.e. their antioxidant activity is increasing.

A differential pulse voltammetric study of (+)-catechin was performed over a wide pH range from 1.1 to 12.5 (Fig. 4), and peaks 1 and 2 were always observed. Comparing the oxidation potentials at different pH provides information on the mechanism of (+)-catechin oxidation and the dependence of the oxidation of (+)-catechin on pH was verified. The strong adsorption of its oxidation product, which blocked the electrode surface, was also observed, since the (+)-catechin oxidation peak 1, as well as the others, decreased drastically in the second scan for all pH values.

The slope of the plot of E_p versus pH was 59 mV per pH unit for peak 1 for all pH range (Fig. 5). This peak corresponds to the oxidation of the 3′,4′-dihydroxyl moiety at B ring (catechol moiety) of (+)-catechin, and the reaction is pH dependent for all pH range studied. The plot shows that the same number of electrons and protons are involved in the oxidation of (+)-catechin, meaning that during the reaction not only electrons but also protons are released from the molecule.

In the plot of E_p versus pH for peak 2 there is a pH dependent range between pH 3 and 9; for pH < 3 or pH > 9 the electron transfer reaction was pH independent, and this peak should be associated with the OH group at position 3 on the C ring. This is in agreement with studies with 3-hydroxyflavone which exhibited the oxidation wave at pH 7 at +0.5 V while the oxidation of 7-hydroxyflavone occurred at higher potentials [24], as well as with the electrochemistry of the 3-OH group relative to hydroxylation at other positions [25].

The occurrence of oxidation peak 3 due to the resorcinol ring A occurs at very high positive potentials and is only observed using differential pulse voltammetry (Fig. 6).

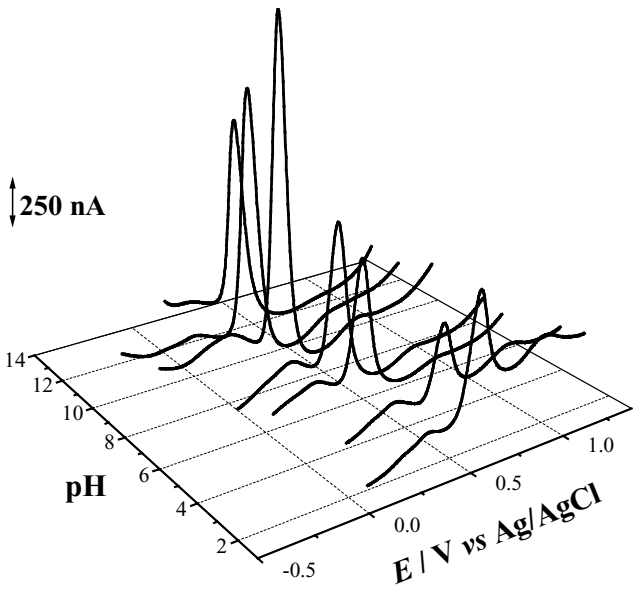

Fig. 4. 3D plot of differential pulse voltammograms of 1 mM (+)-catechin as a function of pH. Scan rate 5 mV s^{-1}.

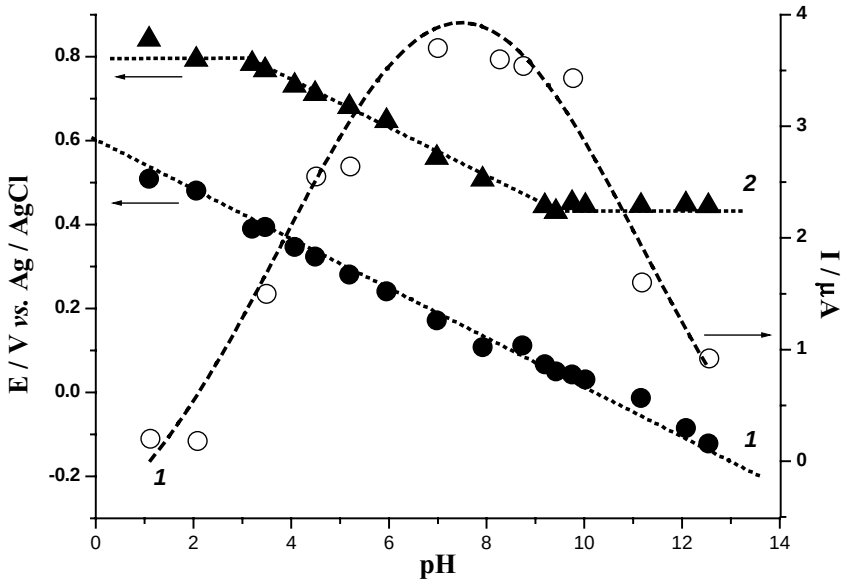

Fig. 5. Dependence of (○) I_p of peak 1, and E_p of (●) peak 1 and E_p of (▲) peak 2 with pH. Concentration of 1 mM (+)-catechin.

Square wave voltammetry was also used as it has the advantage of a greater speed of analysis, a lower consumption of electroactive species in relation to differential pulse voltammetry, and reduced problems with blocking of the electrode surface [26,27]. Square wave voltammetry confirmed the results of differential pulse and cyclic voltammetry, i.e. oxidation peaks 1 and 2, the appearance of significantly lower currents due to adsorption on the second scan, together with a decrease in peak current in more acidic and in more alkaline media. The square wave voltammetry conditions chosen, corresponding to an effective scan rate of 50 mV s^{-1}, led to well-defined voltammograms for all pHs (Fig. 7).

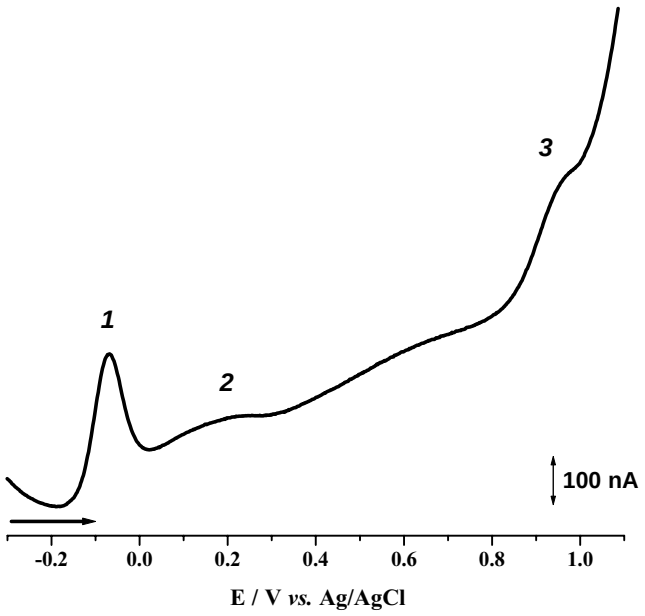

Fig. 6. Differential pulse voltammogram for oxidation of 1 mM (+)-catechin in pH 12.0 KCl/NaOH supporting electrolyte. Scan rate 5 mV s^{-1}.

Another great advantage of the square wave method is the possibility to see during one scan if the electron transfer reaction is or is not reversible. Since the current is simultaneously sampled in both the positive and the negative-going pulses, peaks corresponding to the oxidation or reduction of the electroactive species at the electrode surface are obtained in the same experiment. The reversibility of the oxidation peak 1 of (+)-catechin is clearly shown for all pHs studied (Fig. 7), where the forward and backward currents are similar and the oxidation and reduction peaks occur at the same potential. The second oxidation reaction, peak 2, in (+)-catechin, always shows irreversibility. These results compare very well with those from cyclic and differential pulse voltammetry.

4. Discussion

The first oxidation of (+)-catechin occurs at very low positive potentials which implies a high radical scavenging activity and is a reversible reaction. The hydroxyl group oxidised next was shown to undergo an irreversible oxidation reaction.

The pH-dependent effect on (+)-catechin antioxidant activity is mainly due to an increased radical scavenging ability upon deprotonation. Because deprotonation generally enhances the antioxidant action and because only the ionisation potential (IP), and not the bond dissociation energies (BDE), becomes significantly lower upon deprotonation, it can be concluded that electron donation

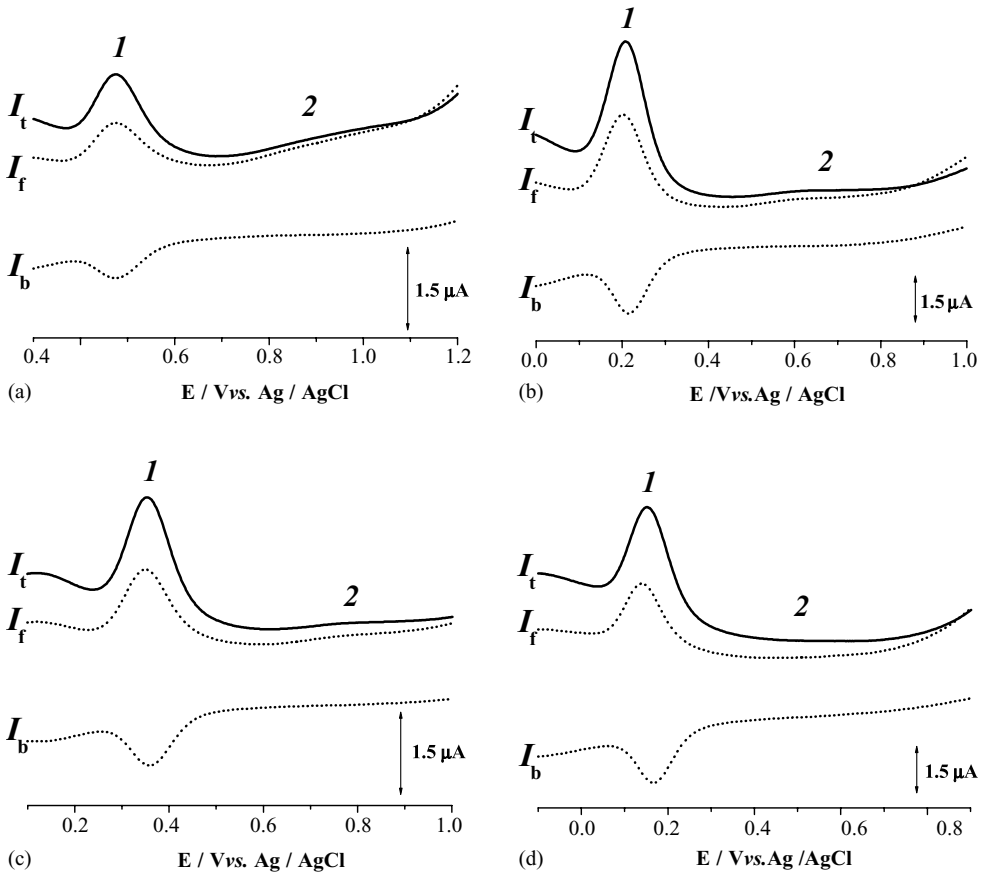

Fig. 7. Square wave voltammograms for oxidation of 1 mM (+)-catechin: (a) pH 2.1; (b) pH 4.5; (c) pH 7.0; (d) pH 8.7. I_t—total current; I_f—forward current; I_b—backward current. Frequency 25 Hz, amplitude 50 mV, effective scan rate 50 mV s^{-1}.

is the dominant mechanism of antioxidant action after deprotonation. Upon deprotonation the radical scavenging capacity increases because electron and not proton donation becomes easier. This suggests that the ease of radical scavenging and also the mechanism of antioxidant activity may change upon deprotonation, and electron donation may be more important for antioxidant action at physiological pH [28].

As shown in Scheme 1, (+)-catechin has two different pharmacophores, the catechol group in ring B and the resorcinol group in ring A and it has also the hydroxyl group at position 3 in ring C. The activity of OH groups in the resorcinol and 3-OH is enhanced by an electron-donating effect of the hydroxyl groups at positions 5 and 7. The hydroxyl groups in ring B are electron-donating and stabilise active intermediates.

The (+)-catechin oxidation proceeds by a consecutive mechanism and is related mainly with the catechol groups in ring B and the three hydroxyl groups in rings A and C present less electroactivity. The oxidation of the catechol moiety, 3′,4′-dihydroxyl electron-donating groups at ring B, occurs first at very low positive potentials corresponding to peak 1. Whilst hydroquinone and catechol have similar potentials, resorcinol is much less readily oxidised, with an oxidation potential ∼350 mV higher then catechol [29].

The current of peak 1 is very high compared with the current of peak 2 and this is in agreement with the higher radical scavenging activity corresponding to the oxidation of the catechol moiety (Fig. 5). The oxidation current is strongly dependent on pH and is higher at neutral pH values corresponding to deprotonation of the hydroxyl moiety, meaning that after deprotonation electron transfer becomes easier, and the mechanism of radical scavenging antioxidant activity of (+)-catechin in the neutral form is increased.

5. Conclusions

The oxidation process of (+)-catechin is complex, pH-dependent, (+)-catechin strongly adsorbs on the electrode surface and the final product is not electroactive and blocks the electrode surface. The first electron transfer reaction of (+)-catechin is reversible over the whole pH range studied. A dependence of peak current on pH was observed showing a maximum around neutral pH values and decreasing in acidic and alkaline solution. The influence of deprotonation of the catechol group is related to the electron/proton donating capacity in (+)-catechin and to its radical scavenging antioxidant activity.

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References

[1] C.A. Rice-Evans, N.J. Miller, G. Paganga, Trends Plant Sci. 2 (1997) 152.
[2] R.A. Larson, Phytochemistry 27 (1988) 969.
[3] J.B. Harborne, C.A. Williams, Phytochemistry 55 (2000) 481.
[4] P. Greenwald, C.K. Clifford, J.A. Milner, Eur. J. Cancer 37 (2001) 948.
[5] C.A. Rice-Evans, N.J. Miller, G. Paganga, Free Radical Biol. Med. 20 (7) (1996) 933.
[6] C.G.M. Heijnen, G.R.M.M. Haenen, J.A.J.M. Vekemans, A. Bast, Environ. Toxicol. Pharmacol. 10 (2001) 199.
[7] B. Yang, A. Kotani, K. Arai, F. Kusu, Anal. Sci. 17 (2001) 599.
[8] B. Halliwell, Free Radical Res. Commun. 9 (1990) 1.
[9] F. Shadidi, P.K.J. Wanasundara, Crit. Rev. Food Sci. Nutr. 32 (1992) 67.
[10] P. Zumman, J.J. Holthuis, Recueil des Travaux Chimiques des Pays-Bas. 107 (1988) 403.
[11] C. Cren-Olivé, P. Hapiot, J. Pinson, C. Rolando, J. Am. Chem. Soc. 124 (2002) 14027.
[12] N.P. Slabbert, Tetrahedron 33 (1977) 821.
[13] S.V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M.G. Simic, J. Am. Chem. Soc. 116 (1994) 4846.
[14] H. Hotta, H. Sakamoto, S. Nagano, T. Osakai, Y. Tsujino, Biochimica et Biophysica Acta 1526 (2001) 159.
[15] S. Chevion, M.A. Roberts, M. Chevion, Free Radical Biol. Med. 6 (2000) 860.
[16] S. Mannino, O. Brenna, S. Buratti, M.S. Cosio, Electroanalysis 10 (1998) 908.
[17] H. Hotta, S. Nagano, M. Ueda, Y. Tsujino, J. Koyama, T. Osakai, Biochim. Biophys. Acta 1572 (2002) 123.
[18] M. Mochizuki, S. Yamazaki, K. Kano, T. Ikeda, Biochim. Biophys. Acta 1569 (2002) 35.
[19] H.M. Merken, G.R. Beecher, J. Agric. Food Chem. 48 (2000) 3.
[20] J.J. Dalluge, B.C. Nelson, J. Chromatogr. A 881 (2000) 411.
[21] H. Nagaoka, S. Toyoshima, K. Takeda, Anal. Sci. 18 (2002) 951.
[22] M. Sano, M. Tabata, M. Suzuki, M. Degawa, T. Miyase, M. Maede-Yamamoto, Analyst 126 (2001) 816.
[23] M. Kumamoto, T. Sonda, K. Takedomi, M. Tabata, Anal. Sci. 16 (2000) 139.
[24] Marian Filipiak, Anal. Sci. 17 (Suppl. i) (2001) 1667.
[25] H.P. Hendrickson, A.D. Kaufman, C.E. Lunte, J. Pharm. Biomed. Anal. 123 (1994) 325.
[26] C.M.A. Brett, A.M. Oliveira Brett, Electroanalysis, Oxford Chemistry Primers, 1998.
[27] C.M.A. Brett, A.M. Oliveira Brett, Electrochemistry: Principles, Methods and Applications, Oxford Science Publications, 1993.
[28] K. Lemanska, H. Szymusiak, B. Tyrakowska, R. Zielinski, E.M.F. Soffers, I.M.C.M. Rietjens, Free Radical Biol. Med. 17 (2001) 879.
[29] S. Steenken, P. Neta, J. Phys. Chem. 86 (1982) 3661.