Antioxidant activity of gallates: an electrochemical study in aqueous media

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Abstract

The electron-donating ability of gallates, which are food and pharmaceutical antioxidants, is quantitatively assessed on the basis of their electrochemical characteristics. Gallic acid and the propyl, *i*-propyl, butyl, *i*-butyl, pentyl and *i*-pentyl gallate derivatives were electrochemically oxidized on the glassy carbon electrode by using differential pulse voltammetry, cyclic voltammetry and hydrodynamic voltammetry on the rotating disk electrode. All the compounds under study were easily oxidized in acidic and neutral solutions. Electrochemical oxidation occurs via two electron-transfer steps; however good resolution for the second wave was obtained only by using hydrodynamic conditions. The oxidation process results to be irreversible, diffusion controlled and pH-dependent. The introduction of the alkyl groups seems to affect the intensities of the semiquinone gallate radicals as can be ascribed from the observed differences in i_d^{II}/i_d^{I} ratio obtained from hydrodynamic voltammetric experiments for the different derivatives. We have found that the intensity of the gallate radicals follows the sequence GA $\geq$ *i*-PG > PG > *i*-BG > BG > *i*-PeG > PeG. From the pH-dependence of the peak current it is possible to affirm that pH 2 is the better condition for the oxidative activity showing that the antioxidant behaviour of these compounds are important in the stomach acid.

Keywords: Gallates; Carbon electrode; Propyl; Rotating disk electrode

1. Introduction

Phenolic antioxidants are chemicals commonly added to food and pharmaceutical products to improve their stability and especially to prevent rancidness in products containing lipids or fats. In recent years the technological and economic requirements of the pharmaceutical and food industries have intensified the use of these compounds. Propyl gallate (PG), its related gallates, and gallic acid are compounds used as antioxidants for the above purposes [1]. Furthermore, some of these compounds have shown other effects such as inhibition of the respiratory chain of the *Trypanosoma cruzi* parasite [2], antimicrobial activity against *Escherichia coli* [3] and toxicity to rat hepatocytes due to mitochondrial disfunction [4]. Moreover, recent results have shown that the antimutagenic properties of some phenolic compounds such as gallates may be due to their inhibiting action on the cytochrome P-450 enzymes [5]. On the other hand, evidence for the formation of the alkyl gallate radicals has also been obtained by electron spin resonance spectroscopy [6].

Probably all of the above properties of these phenolic compounds are related with their redox properties and consequently, a knowledge of the redox behaviour is a very important basis to obtain better explanations of their properties. In spite of this point, there are only a few papers where the electrochemical aspects of gallates and gallic acid have been touched with only one paper devoted to the polarographic behaviour of gallic acid [7]. Other papers are related to analytical aspects to satisfy the requirements to control the amount of gallate additives added to any food or drug. Two of these papers [1,8] are related to electrochemical detection in HPLC for the control of phenolic antioxidants in drugs and foods. Another recent study [9] informs about the voltammetric behaviour of the phenolic antioxidant propylgallate (PG) in aqueous solution at the modified poly(3-methylthiophene)-coated cylindrical carbon fibre microelectrodes.

It is paradoxical that, gallic acid and alkylgallates being polyphenolic compounds of recognized antioxidant capacity,so far there is no systematic study describing their electrooxidative behaviour. The present paper deals with the voltammetric behaviour of a comprehensive series of alkyl gallates and gallic acid in aqueous media at solid electrodes by using, cyclic, differential pulse and hydrodynamic voltammetry. Certainly, if the compounds are used as antioxidants it would be useful to know at what pH they are active, consequently, the present study includes the electrochemical behaviour in a wide range of pH.

2. Experimental

2.1. Materials

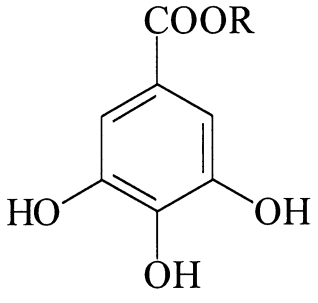
All gallic acid esters, except commercial propyl gallate (PG) (Aldrich®), were synthesized from gallic acid (GA) (Aldrich ®) following a published procedure [10].

Isopropyl gallate (*i*-PG).-m.p.; 125–126°C. IR spectra max (cm^{-1}): 3271 (OH), 297.4 (sp3 CH), 1670 (C=O), 1613.6 (C=C). 1H-NMR spectra: 1.15 (d, 6H, CH3), 4.96 (m, 1H, CH), 6.97 (s, 2H, arom.) 8.0 (s, 3H, OH). Anal. Calc. for C10H1205: C, 56.0 H 5.70 found C, 56.70, H, 5.85. Butyl gallate (BG).-m.p.; 136-137°C. IR spectra max (cm^{-1}); 3332.3 (OH), 2960.8 (sp3 C–H), 1689.8 (C=O), 1615 (C=C).1H-NMR spectra: 0.95 (d, 3H, CH3), 1.62 (m, 2H, CH2-b), 1.80 (m, 2H, CH2-c), 4.25 (t, 2H, OCH2), 7.15 (s, 2H, arom.), 8.25 (s, 3H, OH). Anal. Calc. for C 11H1405 C, 58.40 H 6.24 Found C, 58.55 H 6.40.

Isobutyl gallate (*i*-BG).-m.p.; 126–127.5°C. IR spectra max (cm^{-1}); 3351 (OH), 2961.4 (sp3 C–H), 1689.8 (C=O), 1615.1 (C–C). 1H-NMR spectra: 1.00 (d, 6H, CH3), 2,05 (m, 1H, CH), 4.00 (d, 2H, OCH2), 7.1% (s, 2H, arom.), 8.15 (s, 3H, OH) Anal. Calc.. for C11H1405 C, 58.40 H, 6.24 Found C,58.50 H, 6.21.

Pentyl gallate (PeG).- m.p.; 122-123°C. IR spectra max (cm^{-1}): 3353.3 (OH), 2958.9 (sp3 CH),1694 (C=0),1610.1(C=C). 1H-NMR spectra: 0.95 (t, 3H, CH3), 1,40 (m, 4H, CH2), 1.75 (m, 2H, CH2), 4,20 (t, 2H, OCH2), 7,15 (s, 2H, arom.), 8,20 (s, 3H, OH). Anal Calc. for C12H1605 C, 59.99 H, 6,71 Found C, 59.94 H, 6.70

Isopentyl gallate (*i*-PeG).-m.p.; 139–140°C, IR spectra max (cm^{-1}): 3357.4 (OH), 29576 (sp3 C–H), 1694,5 (C=O), 1610,5 (C=C). 1H-NMR: 0.95 (d, 6H, CH3),



R	Derivatives
H	Gallic Acid
$CH_2CH_2CH_3$	Propyl Gallate
$CH(CH_3)_2$	Isopropyl Gallate
$CH_2CH_2CH_2CH_3$	Butyl gallate
$CH_2CH(CH_3)_2$	Isobutyl Gallate
$CH_2CH_2CH_2CH_2CH_3$	Pentyl gallate
$CH_2CH_2CH(CH_3)_2$	Isopentyl Gallate

Fig. 1. Chemical structure of alkyl gallates.

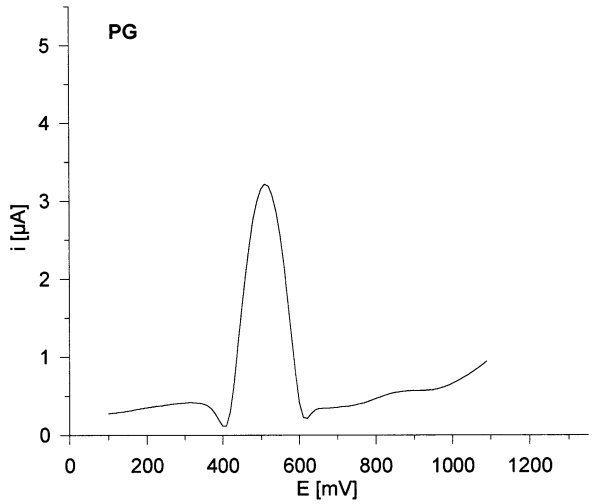


Fig. 2. Differential pulse voltammograms of 1 10^{-4} M solutions of propyl gallate at pH 2 on glassy carbon electrode.

1.65 (m, 1H, CH), 1.80 (q, 2H, CH2), 4.25 (t, 2H, OCH2), 7,15 (s, 2H, arom.), 822 (s, 3H, OH). Anal. Calc.for C12H1605 C, 59.99 H, 6.71 Found C, 59.80 H, 6.88.

Solutions for differential pulse and hydrodynamic voltammetry were prepared by weighing an adequate quantity of gallic acid or gallate compounds in order to obtain a final concentration of 0.1 mM. The gallic acid or gallate compounds were dissolved in 0.4 M Britton Robinson buffer.

Solutions for cyclic voltammetry were prepared by weighing an adequate quantity of gallic acid or gallate compounds in order to obtain a final concentration of lmM, and were dissolved in Britton Robinson buffer with 0.3 M KCl. The pH of the solutions was adjusted using HCl or NaOH. All chemicals used were of analytical-reagent grade, and water was obtained from a Millipore® Milli Q purlfication system.

2.2. Apparatus

A Metrohm® thermostatic cell with three electrodes was employed. A platinum wire and a Ag/AgCI were used as auxiliary and reference electrodes respectively. A glassy carbon electrode was used as working electrode for differential pulse voltammetry. A rotating disk electrode, type EDI with a CONTROVIT rate control unit Tacussel®, equipped with a glassy carbon tip for the rotating disk experiments was used. Electrochemical measurements were carried out in an Inelecsa® assembly similar to that described in a previous paper [11].

Spectrophotometric measurements were carried out in UV-Vis spectrophotometer ATI Unicam® model UV3, using 1-cm quartz cells and equipped with a 486 computer with Vision® acquisition and treatment program.

IR spectra were done in KBr pellets with absorptions expressed per centimetre in a Brucker® FT-JR ISS-66V spectrometer.

All ^{1}H-NMR spectra were recorded at 300 MHz in acetone-d_6 in a Brucker® AMX-300 spectrometer.

Elemental analyses were obtained from a Microanalizer EA-1108 Fisons® model.

3. Results and discussion

The antioxidant action of gallates is expected to be similar to that of other polyphenols such as catechins, which behave as antioxidants by electron donation to free radical oxidants in aqueous solution [12–14]. In order to assess the antioxidant potential properties of gallates, we investigated the availability of these compounds in order to transfer electrons to the electrode.

GA, PG and the five synthesized compounds *i*-PO, BG, *i*-BG, PeG and *i*-PeG (Fig. 1) were subjected to an electrochemical study in the differential pulse, hydrodynamic and cyclic voltammetric modes, with the aim of characterizing their oxidative behaviour.

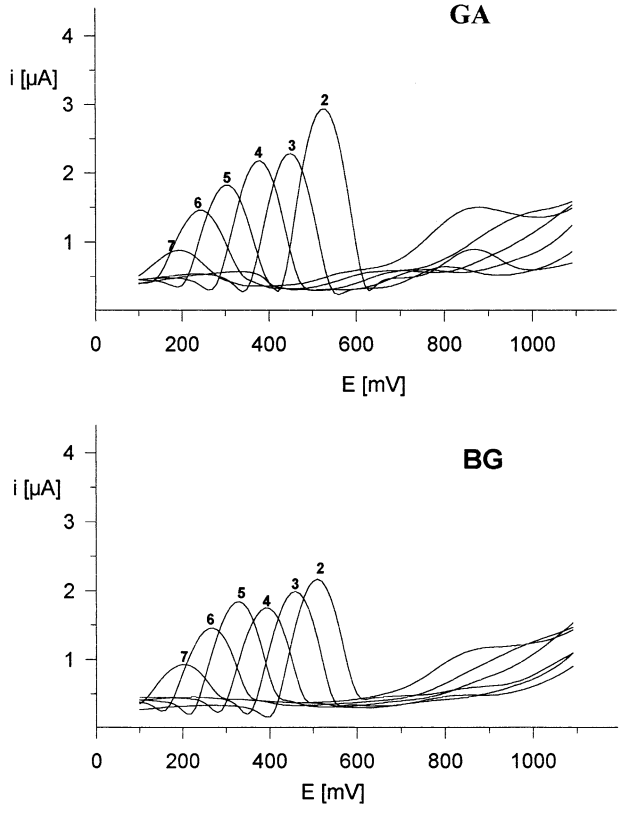


Fig. 3. Effect of pH on the differential pulse voltammograms of gallic acid and butyl gallate.

3.1. Differential pulse voltammetry

All the studied compounds turn out to be easily oxidizable when submitted to voltammetric experiments. Best resolution with the differential pulse mode at the glassy carbon electrode in aqueous media was obtained. Fig. 2 shows the differential pulse voltammogram of one of the compounds under study at pH 2. As can be observed, all the compounds exhibit a very good resolved voltammetric peak at approx. 500 mV. Moreover, for some derivatives a more anodic signal is suggested, but it is very poorly resolved. From the shape of the main peak, we can observe a sharp decrease in the base line before and after the peak. This phenomenon can be ascribed to a strong change in the charging current probably due to adsorption effects. The influence of pH on the voltammetric response was examined for all the gallate derivatives in a Britton Robinson buffer aqueous medium. The pH effect on the differential pulse voltammograms of GA and BG are exemplified in Fig. 3. All the compounds follow a similar behaviour but in PeG and *i*-PeG the ill resolved second peak was not observed. The results obtained clearly show that protons are involved in the oxidation process of all the derivatives. In order to quantify the pH effect we have obtained both potential peak and peak current pH-dependence. The plot E_p versus pH (Fig. 4) shows a linear dependence of the first peak potential between pH 2 and 8. The second peak showed a similar behaviour but the quantification is rather imprecise because the resolution of the peak is very poor. Regarding pH-dependence of the first peak (Fig. 5), a decrease from acidic to neutral or basic media was observed. From the above results, it can be observed that all the compounds show better oxidation activity at pH 2. As the antioxidant behaviour of these compounds are important in stomach acid then the relevance of the behaviour at pH 2 becomes obvious.

Since differential pulse voltammetry (or electrochemical detection in HPLC) might be a convenient analytical technique to quantify these compounds, we also examined the concentration dependence of the peak current (I_p). From data obtained earlier in this study, we chose to work at pH 2.0 with all the gallate derivatives. At this pH we observed a linear relationship between ip and drug concentration in a range going from 5×10^{-6} to 1×10^{-4} M. The regression equation parameters of the above line are shown in Table 1.

Table 1
Regression equation parameters for the linear behaviour between alkylgallate concentration (C) and peak current (I_p)

Alkylgallate	$m \times 10^{-2}$	n	r
GA	0.104	0.373	0.933
PG	0.197	0.817	0.997
i-PG	0.177	0.791	0 983
BG	0.342	0.542	0 999
i-BG	0.246	0 801	0.996
PeG	0.333	0 243	0 993
i-PeG	0.179	0.136	0 997

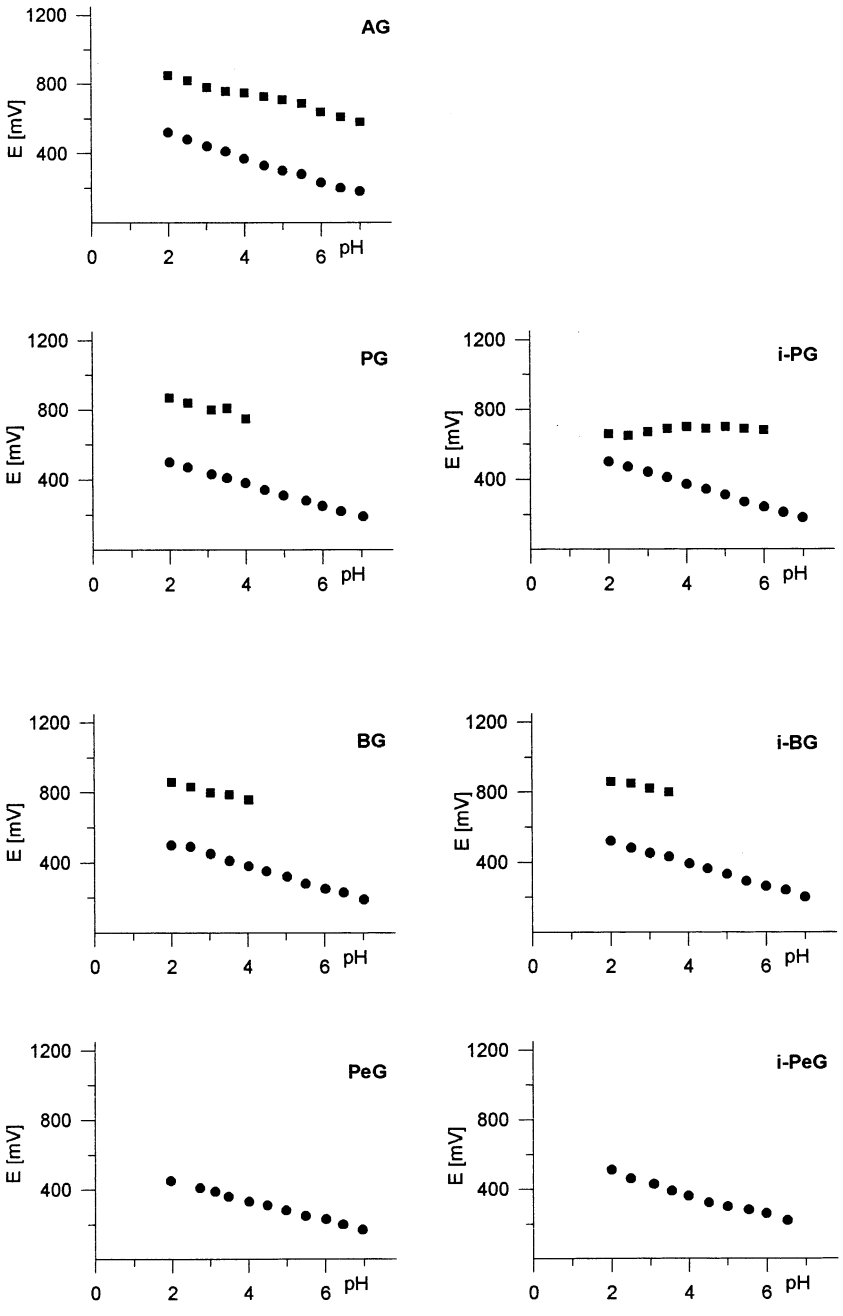


Fig. 4. Potential peak versus pH plot for all the studied alkylgallates.(●) first and (■) second peak.

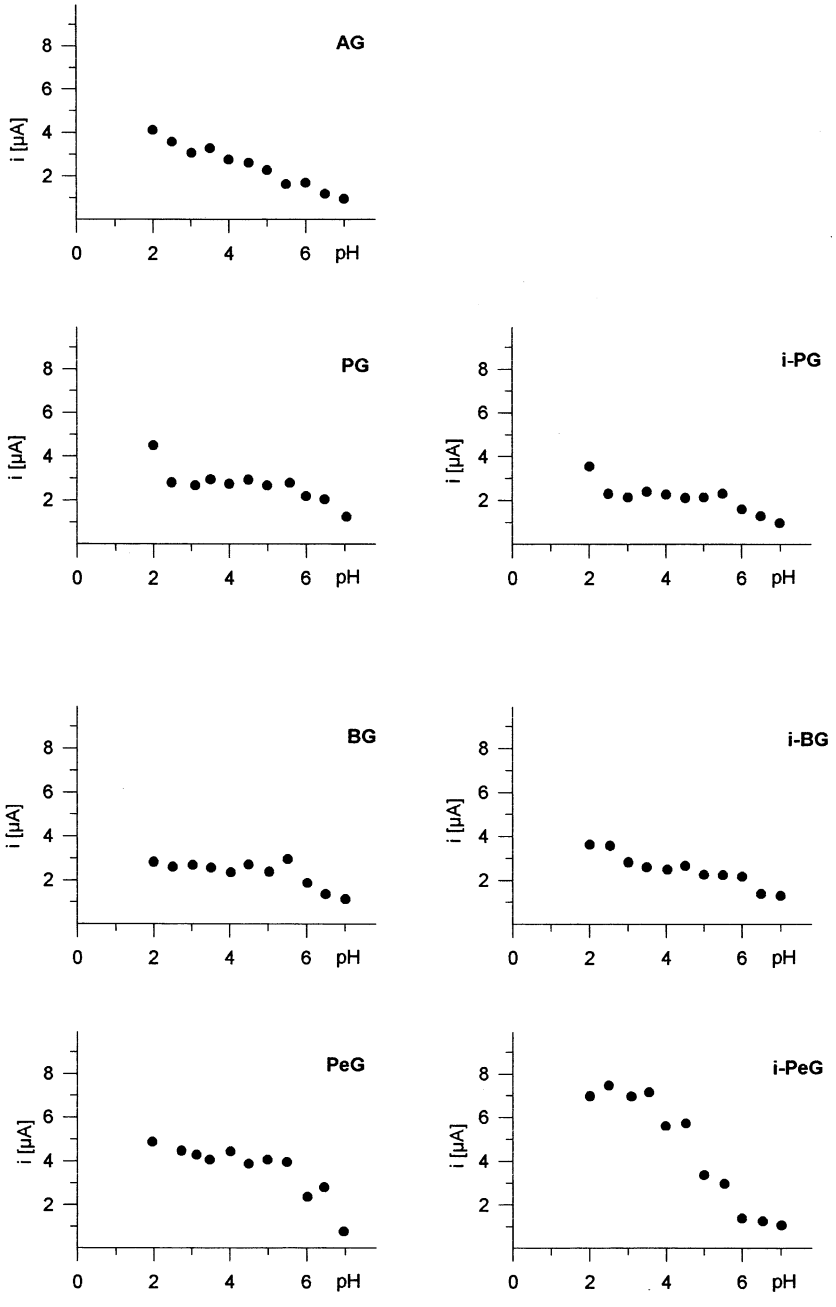


Fig. 5. Current peak versus pH plot for the first peak of all the studied alkylgallates.

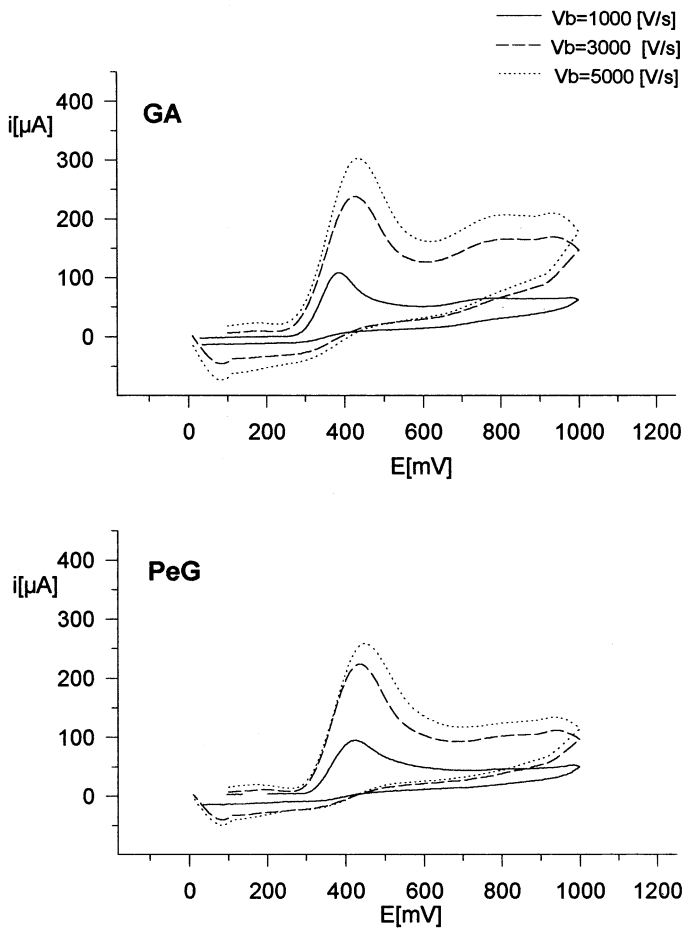


Fig. 6. Cyclic voltammograms of 1 mM solutions of gallic acid and pentyl gallate at different sweep rates.

3.2. Cyclic voltammetry

Cyclic voltammetric experiments showed a single oxidation peak for all seven gallate derivatives. On the reverse sweep, no distinct reduction wave was observed, showing that gallates derivatives are irreversibly oxidized at the GCE. Fig. 6 shows the cyclic voltammograms of two of the compounds under study at three different sweep rates. As seen in the GA cyclic voltammograms, a second oxidation peak is clearly distinguished at more anodic potentials. However, for the other compounds this second peak is only weakly suggested at higher sweep rates. From the cyclic voltammetric study at pH 2.0 and different sweep rates for all the seven gallate derivatives, it may be seen that: (a) the dependence of the peak current (I_p) on the square root of the scan rate ($v^{1/2}$) was linear over the whole scan rate range tested

(0.1–5 $V^{1/2}$). (b) The current function $I_p/v^{1/2}$ remained virtually constant over such scan rate range (Table 2) (c) the log I_p versus log v plot is linear, with a slope value of approximately 0.5 (Table 2). (d) Furthermore, from the relation for the width of the peak ($E_p - E_{p/2}$) [15] we have obtained the transfer coefficient for all the oxidation processes (Table 2). All these results clearly prove that the oxidation current was purely diffusion-controlled and all the compounds follow a similar pathway and kinetics.

3.3. Hydrodynamic voltammetry

In contrast with the above results obtained by voltammetry on stationary electrodes, when the rotating disk electrode was used, we clearly distinguished two waves for each studied compound. However, as seen in Fig. 7, the resolution of the second wave diminished notoriously with the increase in the carbon atom number of the ester derivative. The effect of increasing the rotating disk electrode rate is shown in Fig. 8. All the compounds gave a very good resolved first wave with a well defined limiting current, which was proportional to the square root of the rotation speed (between 200 and 3000 rpm) with a zero or near zero intercept. Examples of Levich plots [16] are given in Fig. 9. The linear behaviour of these plots suggests that the electrode process is diffusion controlled without kinetic complications. Owing to the rotation of the electrode, a strong convective force is imposed on the solution and in comparison other forces that may influence the solution transport are small and can be neglected. Furthermore, the diffusion layer thickness for a rotating electrode is small compared with that of a stationary electrode, and taken together, these characteristics result in an experimental set up that is relatively insensitive to the nature of the surroundings [17]. This may be the explanation of obtaining a better resolution for the second electron transfer than one observed above on stationary electrodes.

From the electrochemical results we postulate that gallate derivatives oxidize in acidic solutions via two electrons, two protons, according to Scheme 1. This scheme is proposed only for the time schedule of the voltammetric experiments. The postulated Scheme 1 is based on the well described route for the anodic oxidation of phenols, hydroquinones and derivatives [18]. The existence of the alkyl gallate

Table 2
Cyclic voltammetric kinetic parameters obtained for 1×10^{-3} M alkylgallate solutions at pH 2

Alkylgallate	$I_p/v^{1/2}$ ($\mu A/mV^{1/2}$ $s^{1/2}$)	δlog ip/δ 1og v	αn
GA	4.44	0 49	1.1
PG	4.88	0.52	1.1
i-PG	3 37	0.52	1.3
BG	4.56	050	1 1
i-BG	5.89	0.57	1.4
PeG	4.45	0.56	1.1
i-PeG	4.88	0.53	1.2

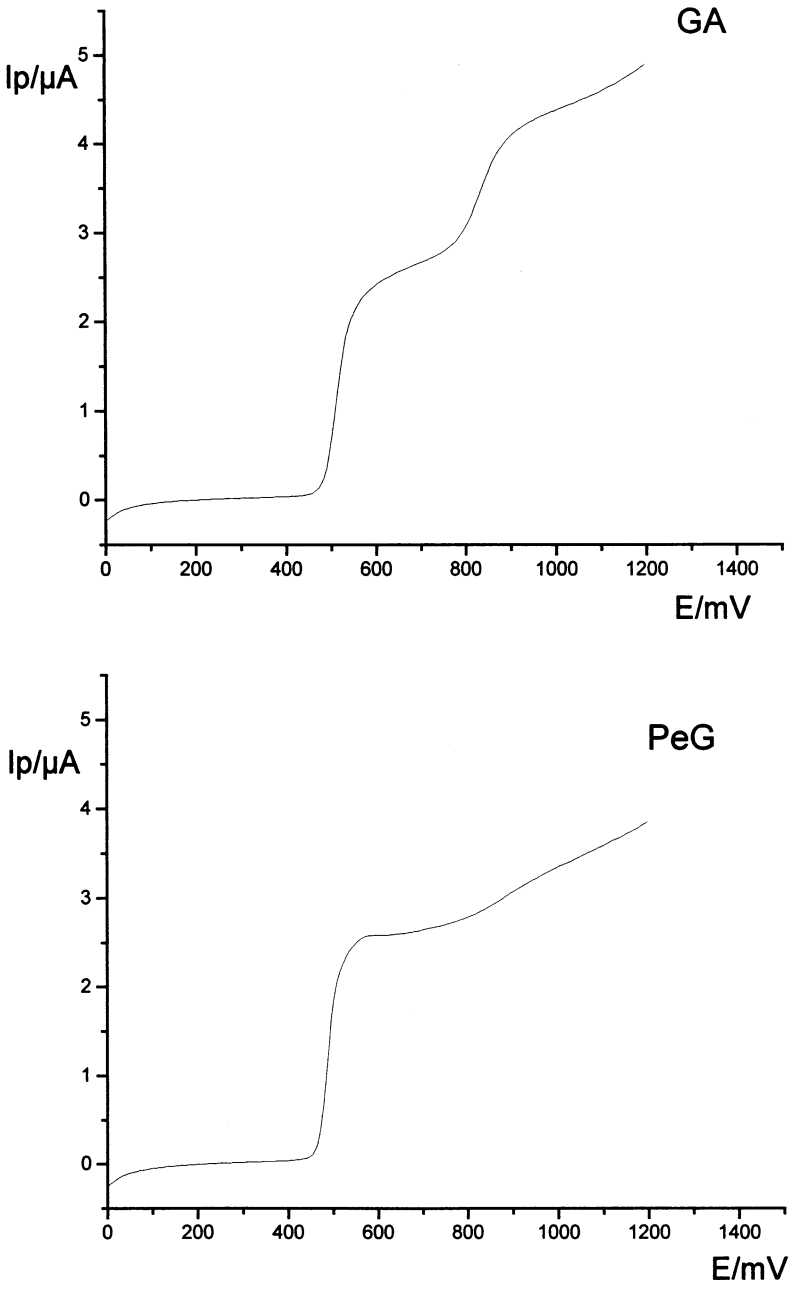


Fig. 7. Hydrodynamic voltammograms of gallic acid and pentyl gallate on the glassy carbon rotating disk electrode at pH 2.0 and 200 rpm.

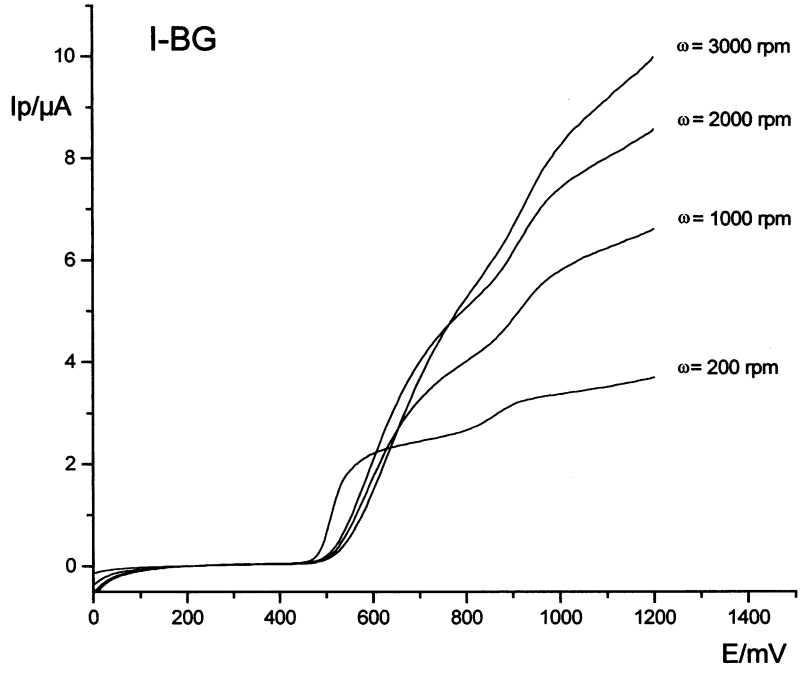


Fig. 8. Hydrodynamic voltammograms of *i*-BG at different rotation rates.

radical species, as proposed in the first de-electronation step reaction, has been previously proved by ESR measurements [6]. Furthermore, the resonance structures of the semiquinone radical have also been proposed for di-catechols [19] with strong oxidative character.

From our results we can conclude that the introduction of alkyl groups did not greatly influence mainly the energetics of the electron transfers as can be ascribed from the similarity of the $E_{1/2}$ values for all the derivatives. This effect is similar for both waves as can be observed in Table 3. However, the main difference observed between the studied compounds was related to the intensity of the second wave (i_d^{II}). When stationary electrodes were used, this second signal had very low intensity and consequently, a very poor resolution. However, with the hydrodynamic method it was possible to quantify both waves. From Table 3 we can observe that as the number of C atoms of the ester alkyl chain increases the intensity of the second wave diminishes compared with the first. This fact can be ascribed to a decrease in the interface concentration of the semiquinone radical due to its instability. This effect was observed both for lineal chain or branched chain substituents in which the sequence of decrease of the intensity in semiquinone radical is given by GA > PG > BG > PeG and *i*-PG > *i*-BG > *i*-PeG. When the lineal alkyl radical is compared with branched alkyl radicals for a same number of C atoms, it can be concluded that chain branching permits minor decreases in the second wave, therefore, more stable semiquinone radicals being obtained.

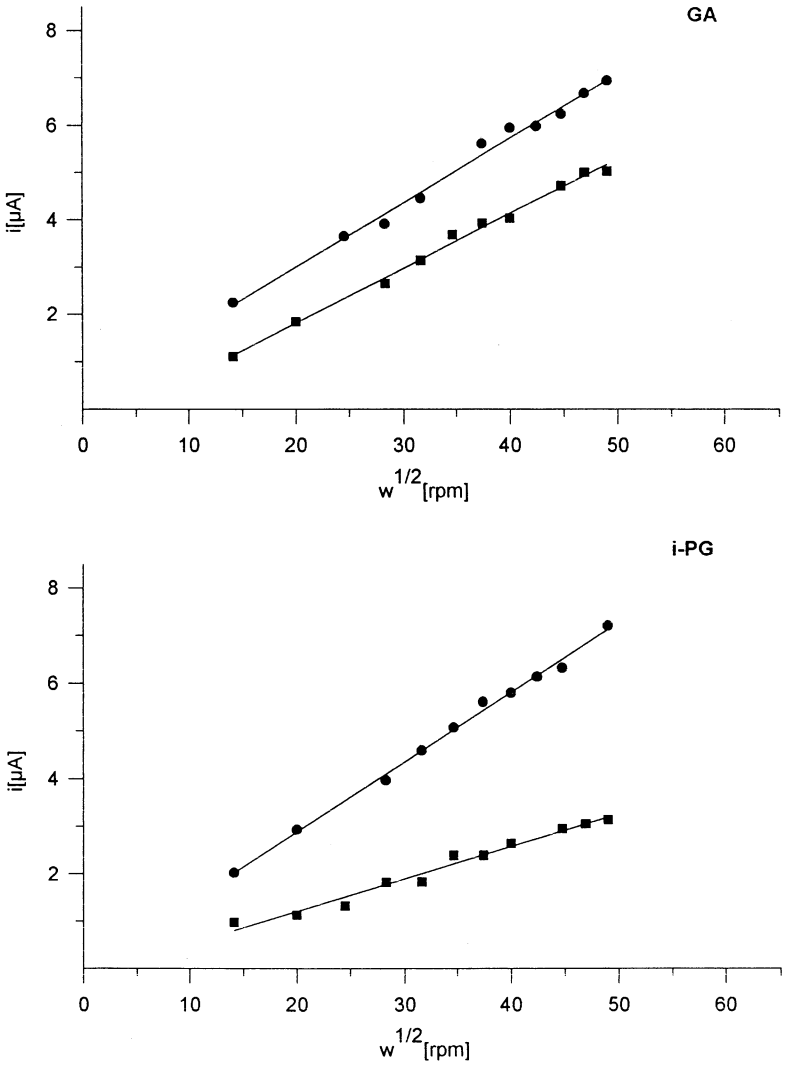
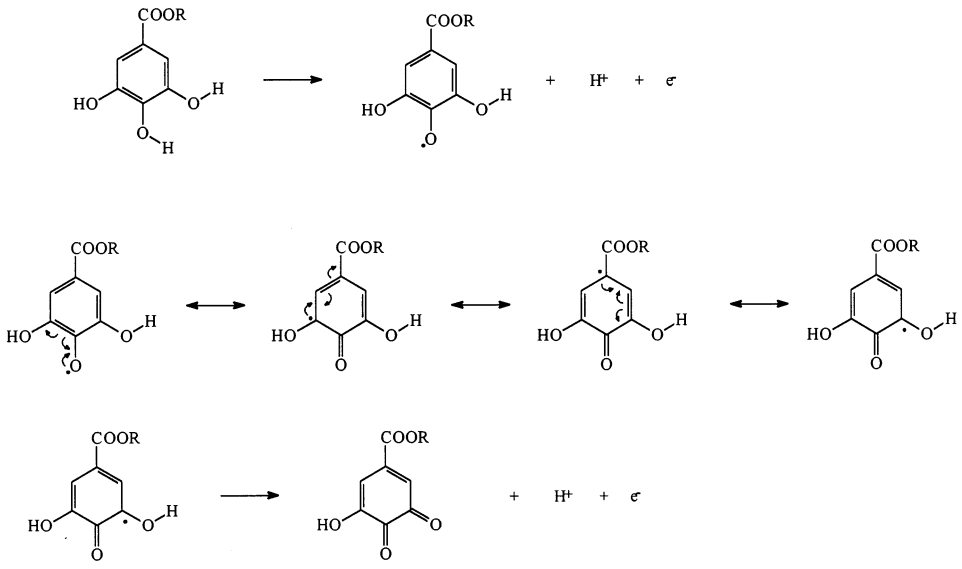


Fig. 9. Levich plots for first and second waves for GA and PG (●) first and (■) second peak.

A recently published paper [20] has concluded that GA induced apoptotic cell death in human promyelocytic leukemia HL-60 cells.But, the effect of GA was significantly reduced by blocking the free hydroxyl or carboxyl group with acetyl, methyl, ethyl, *n*-propyl or isoamyl group. This was paralleled with the decrease in the intensity of both gallate radical and oxidation activity. Our results are in agreement with the above findings in the sense that the introduction of alkyl groups to the carboxyl group of the GA produced a decrease in the intensity of the gallate radicals, as observed in the decrease of the voltammetric signal that corresponds to the one-electron oxidation step of the semiquinone radical.



Scheme 1. Overall oxidation pathway of alkylgallate derivatives.

Table 3
Half-wave potentials and limiting current for the two waves of 1×10^{-4} M gallate derivatives solutions obtained by hydrodynamic voltammetry on the rotating disk electrode.pH 2, 200 rpm.

Alkylgallate	$E^{I}_{1/2}$ (V)	$E^{II}_{1/2}$ (V)	i^{I}_{d} (μA)	i^{II}_{d} (μA)	i^{II}_{d}/i^{I}_{d}
GA	0.52	0.84	2.25	1.11	0.49
PG	0.51	0 85	2.16	0 72	0.33
i-PG	0.53	0.87	2 02	0.98	0.49
BG	0.52	0 88	2.62	0.74	0.28
i-BG	0.52	0 86	2.20	0.68	0.30
PeG	0.49	–	2.40	–	–
i-PeG	0 50	0 87	2.50	0.64	0 25

Acknowledgements

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