Electrochemical Behaviour of Isoflavones Genistein and Biochanin A at a Glassy Carbon Electrode

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Abstract
The electrochemical behaviour of genistein and biochanin A was studied at a glassy carbon electrode by cyclic, differential pulse and square wave voltammetry. Genistein undergoes three irreversible, pH dependent oxidation reactions with the transfer of one electron and one proton from each hydroxyl group. The formation of two electroactive products that undergo reversible redox reactions was observed. Biochanin A undergoes two irreversible, pH dependent reactions due to the oxidation of the two hydroxyl groups. The electrochemical behaviour of the chemical analogue daidzein was also investigated. The electroactive centres of genistein and biochanin A were identified and their oxidation mechanisms discussed.

Keywords: Genistein, Biochanin A, Daidzein, Kinase inhibitor, Glassy carbon electrode

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1 Introduction

Flavonoids are a group of polyphenolic compounds that occur as intrinsic components of fruits, vegetables, and beverages [1,2]. The chemical structure of flavonoids presents two aromatic rings A and B, linked together by an oxygenated heterocycle ring C. Variations on the position of rings A and B relative to ring C yield several classes of compounds such as flavones, isoflavonoids, and neoflavonoids. Within these classes, flavonoids can undergo additional modifications, such as methylation, glycosylation, and dimerization, among others [1,2].

Isoflavones represents a subgroup of flavonoids commonly found in the soy beans, chick pea, and peanuts. The most known isoflavones are genistein, biochanin A and daidzein, Scheme 1, which upon consumption are absorbed into the bloodstream, thus exerting biological effects [3].

Several studies examined the association between the intake of foods rich in polyphenols and diseases [4,5]. Genistein, biochanin A and daidzein have many important health benefits lowering the incidence of cardiovascular diseases [6], preventing osteoporosis [7], and attenuating neurodegenerative effects [8], among others [1,2]. The important biological effect of isoflavones consumption have been elucidated with respect to their anticancer properties [9,10]. It has been shown that both genistein and biochanin A inhibits the growth of breast and prostate cancer cells in vitro [11]. The knowledge regarding their effects is still in progress and clinical trials using isoflavones supplementation are being conducted in patients with cancer [12,13].

The mechanisms through which these compounds exert their actions are not well understood but several studies have reported inhibitory activity against protein-tyrosine kinase (PTK), which attenuates the growth of cancer cells by inhibiting PTK-mediated signalling mechanisms [14–16]. On the other hand, most pharmacological and nutraceutical effects of isoflavones have been attributed to antioxidant mechanisms [17,18] which protects cells against reactive oxygen species by scavenging free radicals, inhibiting the expression of stress–response related genes, thus reducing carcinogenesis. However, as redox agents, isoflavones can play a dual role of antioxidant and pro-oxidants, so an unbalanced diet can lead to negative effects [19]. Hence, the knowledge of the oxidation mechanisms is crucial to ascertain the influence of the redox behaviour, on the chemical, pharmacological and nutritional properties of genistein.

Although the electrochemical behaviour of daidzein has been reported in detail [20], the redox mechanisms of genistein and biochanin A have not been fully characterised [21–23]. In fact, most of the studies concerning genistein and biochanin A dealt with their analytical determination by chromatography with electrochemical detection [22–25]. Thus, the aim of this work is to investigate the oxidation of genistein using a glassy carbon electrode and electrochemical techniques, cyclic, differential pulse and square wave voltammetry. In order to understand and explain genistein oxidation mechanism, its electrochemical

behaviour was compared with chemical analogues such as biochanin A and daidzein.

The electrochemical methods are a promising tool for analytical purposes, especially in the case of electroactive species, and the study of isoflavones redox mechanisms is useful on the quality control of foodstuffs containing such relevant biomarkers [26,27]. Furthermore, the metabolites formed are the key to understand the beneficial effects of isoflavones since it is the metabolite, rather than the parent compound, to which cells are predominantly exposed.

2 Experimental

2.1 Materials and Reagents

Genistein, biochanin A and daidzein were obtained from Extrasynthèse (Genay, France) and used without further purification. Stock solutions of 5 mM were prepared in ethanol-deionised water (50:50, v/v) and stored at 4 °C. Solutions of different concentrations were prepared by dilution of the appropriate quantity in supporting electrolyte.

All supporting electrolyte solutions (Table 1) were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leq 0.1\ \mu S\,cm^{-1}$).

2.2 Voltammetric Parameters and Electrochemical Cell

Voltammetric experiments were carried out using an Ivium potentiostat running with Ivium software version 2.038, Ivium Technologies, The Netherlands. Measurements were carried out using a three-electrode system in a 0.5 mL one-compartment electrochemical cell (Cypress System Inc., USA). Glassy carbon electrode (GCE, $d=$ 1.0 mm) was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 mol L^{-1} KCl) reference electrode.

The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

Table 1. Supporting electrolytes, 0.1 M ionic strength.

pH	Composition
2.0	HCl + KCl
3.4	HAcO + NaAcO
4.3	HAcO + NaAcO
5.6	HAcO + NaAcO
6.0	$NaH_2PO_4 + Na_2HPO_4$
7.0	$NaH_2PO_4 + Na_2HPO_4$
8.0	$NaH_2PO_4 + Na_2HPO_4$
9.2	$NH_3 + NH_4Cl$
10.0	KCl + NaOH
12.0	KCl + NaOH

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 100 ms, potential step 2 mV and a scan rate of 5 mV s^{-1}. For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 25 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 50 mV s^{-1} were used.

The GCE was polished using diamond particles of 3 μm (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammograms were obtained. This procedure ensured reproducible results.

2.3 Acquisition and Presentation of Voltammetric Data

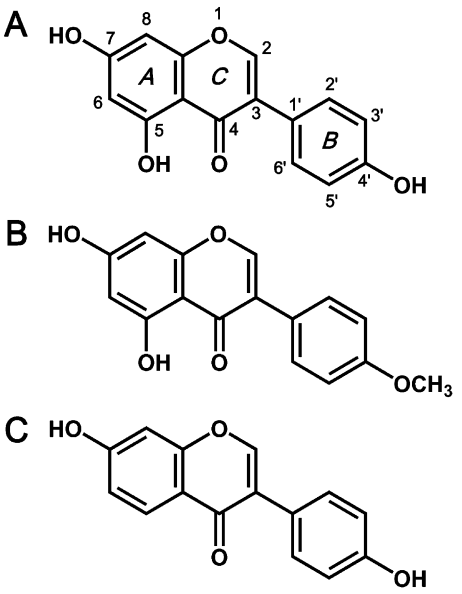
All the voltammograms presented were background-subtracted and baseline-corrected using the automatic function included in the Ivium software. This mathematical treatment improves the visualisation and identification of peaks over the baseline without introducing any artefact, although the peak intensity is, in some cases, reduced (< 5%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all plots were determined from the original untreated voltammograms after subtraction of the baseline.

3 Results

3.1 Cyclic Voltammetry

Genistein is an isoflavone that contains three hydroxyl groups: one at position 4′ in the B ring and two in the A ring at positions 5 and 7 forming a resorcinol type structure (Scheme 1 A).

On the first cyclic voltammogram recorded in 50 μM genistein in pH 4.3 (Figure 1 A) three anodic peaks occurred: Peak 1_a at $E_{p1a} = +0.68$ V, Peak 2_a at $E_{p2a} = +0.85$ V, and Peak 3_a at $E_{p3a} = +1.05$ V. Reversing the scan direction, on the negative-going scan of the first voltammogram, two small cathodic peaks: Peak 4_c, at $E_{p4c} = +0.21$ V, and Peak 5_c, at $E_{p5c} = +0.09$ V, appeared. These peaks are due to the reduction of genistein oxidation products formed at the electrode surface during the first voltammetric scan. On the second voltammogram in the same conditions without cleaning the electrode surface the corresponding anodic Peaks 5_a, at $E_{p5a} = +0.25$ V, and Peak 4_a, $E_{p4a} = +0.40$ V were observed (Figure 1 A). The decrease of the anodic Peaks 1_a, 2_a and 3_a upon successive voltammograms was due to the adsorption of genistein and/or its oxidation products at the GCE surface.



Scheme 1. Chemical structures of: A) genistein, B) biochanin A, and C) daidzein.

A new experiment was carried out in the same conditions but the scan direction was reversed at +0.80 V, after the occurrence of Peak 1_a but before Peak 2_a (Figure 1 A). In these conditions Peaks 5_a and 5_c occurred showing that they are related to the product formed after genistein oxidation at Peak 1_a. Recording successive scans in these conditions the current of Peaks 5_a–5_c increased with the number of scans.

The effect of scan rate on the oxidation of genistein was also evaluated (Figure 1 B). Increasing the scan rate the potentials of all peaks were shifted to more positive values. Peak currents were directly proportional to scan rate indicating the adsorption of genistein and its oxidation products at the electrode surface.

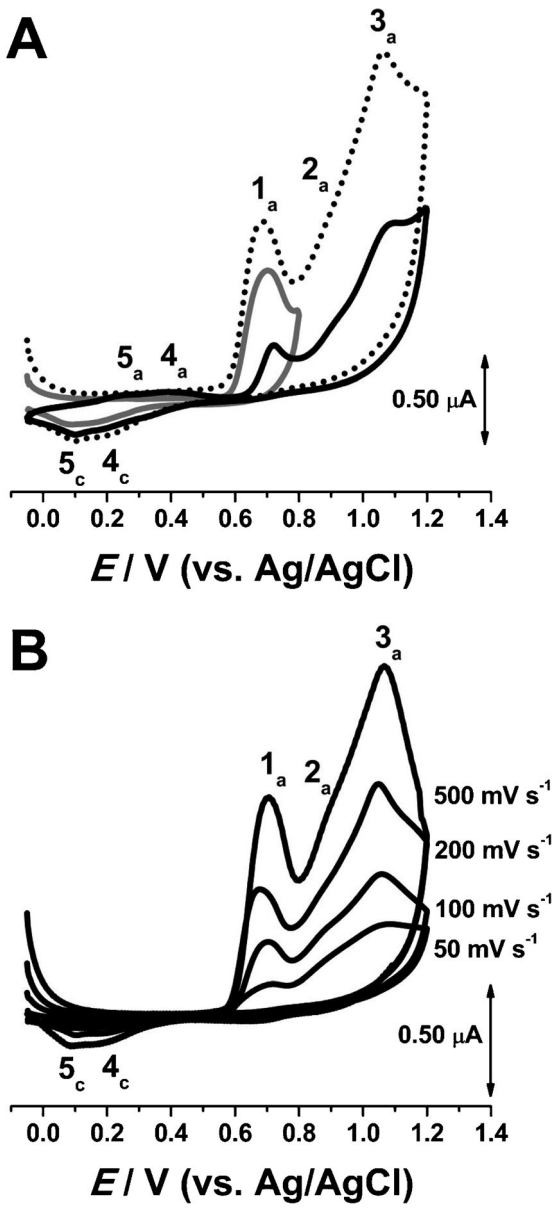


Fig. 1. Cyclic voltammograms base-line subtracted in 50 μM genistein in pH 4.3: A) (dotted curve) first and (black curve) third scans between −0.05 and +1.20 V and (grey curve) first scan between −0.05 and +0.80 V at $v = 200\ \mathrm{mV\,s^{-1}}$ and B) first scan at different *v*.

3.2 Differential Pulse Voltammetry

3.2.1 Genistein

The pH effect on the electrochemical oxidation of genistein was investigated over a wide pH range from 2.0 to 12.0, using different voltammetric techniques.

DP voltammetry allowed lower detection limits and a better visualisation of all redox processes. DP voltammograms were recorded in solutions of 5 μM genistein in electrolytes with different pH values (Figure 2 A).

For pH < 7.5 three consecutive, pH dependent charge transfer reactions correspondent to Peaks 1_a, 2_a and 3_a were observed. The peak potentials turned less positive with increasing the pH of the supporting electrolyte. The relationships were linear and the slope of the lines were −60 mV per pH unit (Figure 2 B) in agreement with the transfer of the same number of electrons and protons. The width at half-height of the peaks was around 90 mV, close to the theoretical value for the transfer of 1 electron.

For pH > 7.5 Peaks 1_a and 2_a occurred. The potentials of Peaks 1_a and 2_a did not depend on the pH of the supporting electrolyte for pH > 7.5 and for pH > 9.2, respectively (Figure 2 B). This is specific to electrochemical reactions that involve the transfer of electrons after chemical deprotonation. Thus, the genistein pK_a values of approximately 7.5 and 9.2 were determined.

The current of Peak 1_a showed higher values for pH < 4.3 while for other pH values the current got decreased. This effect is explained considering that the current registered upon application of a potential step is directly pro-

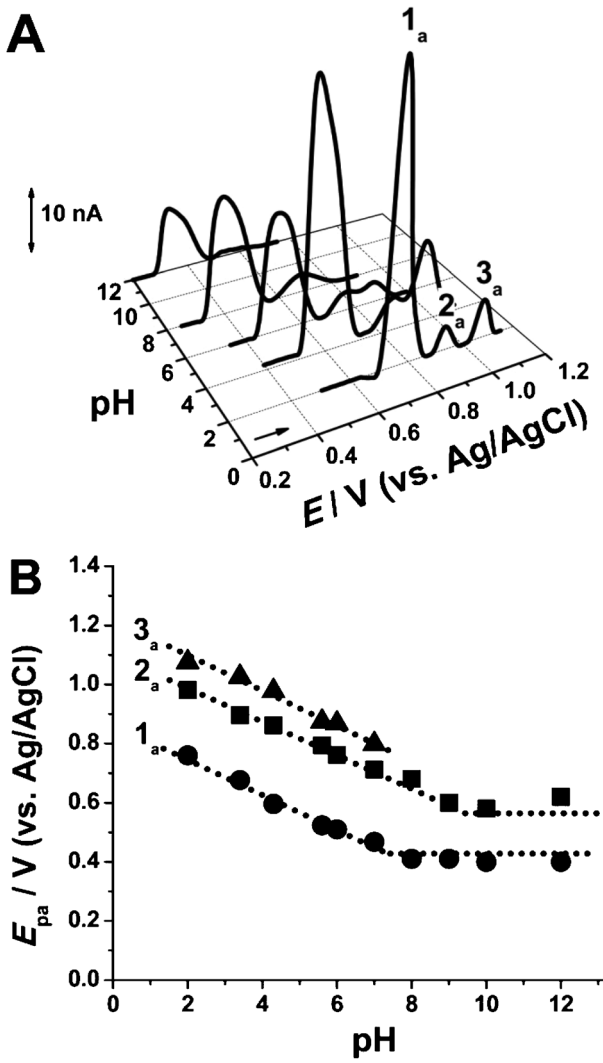


Fig. 2. A) 3 D plot of DP voltammograms baseline-corrected in 5 μM genistein function of the pH of the supporting electrolyte. B) Plot of E_{pa} of Peaks (●) 1_a, (■) 2_a and (▲) 3_a vs. pH.

portional with the rate constant of the heterogeneous electrochemical reaction [28–30]. Hence, the variation of Peak 1_a current is due to the variation of the rate constant of the heterogeneous genistein oxidation with the pH of the supporting electrolyte.

On the other hand, the pH dependent adsorption of genistein and its oxidation products can also lead to the variation of the peak currents. In fact, with increasing pH, genistein and its oxidation products became ionised (deprotonated) which alters their hydrophobicity and consequently their adsorption on the GCE surface [28,29].

Consecutive DP voltammograms were also recorded in solutions of genistein in electrolytes with different pH values. On the first DP voltammogram in pH 7.0, the three consecutive charge transfer reactions correspondent to Peaks 1_a, 2_a and 3_a were observed (Figure 3A and Table 2).

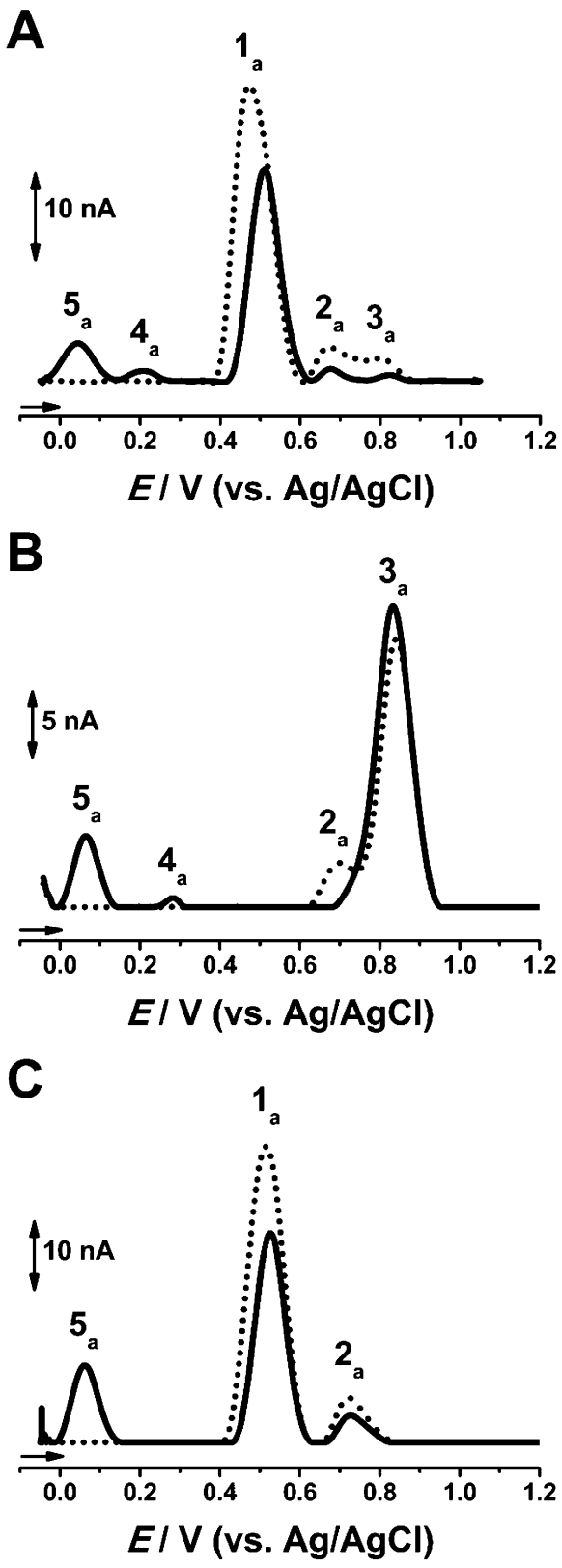


Fig. 3. DP voltammograms baseline-corrected in: A) 5 μM genistein, B) 10 μM biochanin A and C) 5 μM daidzein in pH 7.0; (dotted curves) first and (solid curves) second scans.

Table 2. Oxidation peak potentials in pH 7.0 according to DP voltammograms in Figure 3.

Compound	Oxidation potential (V)				
	Peak 1_a	Peak 2_a	Peak 3_a	Peak 4_a	Peak 5_a
Genistein	0.48	0.67	0.79	0.21	0.05
Biochanin A	–	0.69	0.82	0.23	0.06
Daidzein	0.50	0.72	–	–	0.06

In successive DP voltammograms, Peaks 4_a and 5_a due to genistein oxidation products appeared at lower potential values and their currents increased with the number of scans (Figure 3A and Table 2). The Peaks 1_a, 2_a and 3_a current decreased in successive DP voltammograms and their potential shifted due to the adsorption of genistein oxidation products, on the GCE surface, reducing the available electrode surface area.

The adsorption of the genistein oxidation product at the GCE surface was confirmed when, at the end of several DP voltammograms in the solution, the electrode was washed with a jet of deionised water and transferred to the supporting electrolyte. The DP voltammogram obtained in these conditions (not shown) presented both Peaks 4_a and 5_a corresponding to the genistein oxidation products.

The genistein oxidation products undergo pH dependent redox reactions. The second DP voltammograms in 5 μM genistein in different electrolytes (Figure 4A) showed that increasing the pH Peaks 4_a and 5_a occurred at less positive potentials.

For pH < 9.2, the dependence was linear and the slopes −60 mV per pH unit (Figure 4B). Considering the width at half-height of the peaks of about 55 mV, the oxidation of genistein redox products involved the transfer of two electrons and two protons. For pH > 9.2, Peaks 4_a and 5_a potential did not depend on the pH (Figure 4B) showing that the correspondent reactions involve only the transfer of two electrons.

On the other hand, it has been observed that Peaks 4_a and 5_a currents also varied with the pH (Figure 4B). For Peak 4_a higher currents occurred in alkaline electrolytes while Peak 5_a current has shown higher values in mild acid media. This effect is due to the pH dependent formation rate and adsorption of these products at the GCE surface [30].

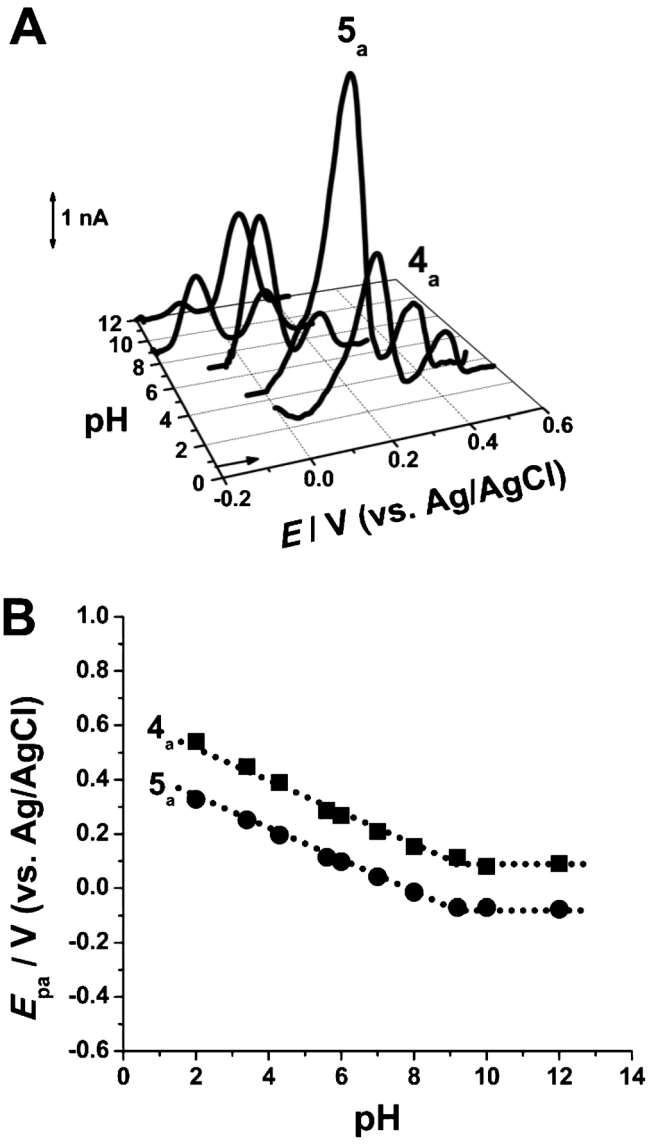


Fig. 4. A) 3 D plot of DP voltammograms (second scan) baseline-corrected in 5 μM genistein function of the pH of the supporting electrolyte. B) Plot of E_{pa} of Peaks (●) 5_a and (■) 4_a of genistein oxidation products vs. pH.

3.2.2 Biochanin A

Biochanin A is similar to genistein but the hydroxyl in the B ring is substituted by a methoxy group (Scheme 1B).

DP voltammograms were recorded in solutions of 10 μM biochanin A in electrolytes with different pH values (Figure 5A).

For acid electrolytes three consecutive charge transfer reactions at Peaks 2_a, 3_a and 6_a were observed, whereas for pH > 6.0 Peaks 2_a and 3_a occurred (Figure 5A and B).

In electrolytes with pH < 9.2, all peaks were pH dependent (Figure 5B).

The potential of Peaks 2_a and 3_a turned less positive with increasing pH. The relationships were linear with slopes of −60 mV per pH unit and the width at half-height of the peaks around 90 mV, in agreement with the transfer of 1 electron and 1 proton.

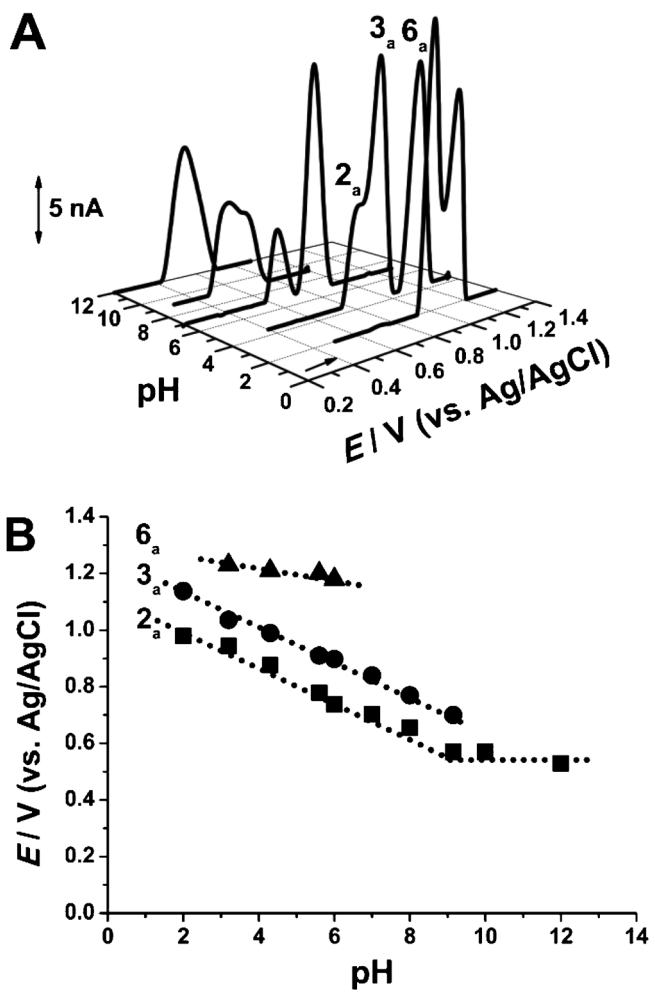


Fig. 5. A) 3 D plot of DP voltammograms baseline-corrected in 10 μM biochanin A function of the pH of the supporting electrolyte. B) Plot of E_{pa} of Peaks (■) 2_a, (●) 3_a and (▲) 6_a of biochanin A vs. pH.

The potential of Peak 6_a decreased linearly with increasing pH. The slope of the line was −30 mV per pH unit, showing that the mechanism of this oxidation process involves a number of electrons that is double the number of protons.

For pH > 9.2, Peak 2_a did not depend on the pH (Figure 5B). In these conditions, the oxidation of biochanin A involved only the transfer of one electron and no proton, allowing the determination of biochanin A pK_a ≈9.2.

Consecutive DP voltammograms were also recorded in solutions of biochanin A in electrolytes with different pH values. On the first DP voltammogram in pH 7.0 two consecutive charge transfer reactions correspondent to Peaks 2_a and 3_a were observed (Figure 3B and Table 2). In successive DP voltammograms, Peaks 4_a and 5_a appeared at lower potentials and their currents increased with the number of scans (Figure 3B and Table 2). These peaks are due to the formation of electroactive biochanin A oxidation products.

3.2.3 Daidzein

Daidzein contains two hydroxyl groups: one in the B ring at position 4′ as in the case of genistein and the second in the ring A at position 7 (Scheme 1C).

The electrochemical oxidation of daidzein has been also studied in order to identify the origin of genistein oxidation peaks. Daidzein underwent oxidation in two consecutive steps. On the first DP voltammogram in 5 μM daidzein in pH 7.0, Peak 1_a and Peak 2_a occurred (Figure 3C and Table 2). Recording successive voltammograms, a new oxidation Peak 5_a appeared. This peak was due to the oxidation of daidzein oxidation product adsorbed at the GCE surface.

Previous studies [20] have shown that daidzein oxidation is pH dependent and both steps involve the transfer of 1 electron and 1 proton.

3.3 Square Wave Voltammetry

Successive SW voltammograms were recorded in a solution of 5 μM genistein in pH 7.0, Figure 6. On the first scan the anodic Peak 1_a at $E_{p1a} = +0.51$ V, Peak 2_a at $E_{p2a} = +0.72$ V and Peak 3_a at $E_{p3a} = +0.82$ V, occurred Figure 6A. By plotting the forward and backward components of the total current, the quasi-reversibility of genistein oxidation at Peak 1_a was observed. Peaks 2_a and 3_a corresponded to irreversible processes since only anodic currents occurred on the forward component and no cathodic correspondent on the backward one (Figure 6A).

By increasing the number of scans in solution, without cleaning the GCE surface, Peaks 5_a and 4_a appeared at $E_{p5a} = +0.07$ V and at $E_{p4a} = +0.24$ V (Figure 6B). The deconvolution of the total current recorded in has shown the reversibility of Peaks 5_a and 4_a since by plotting the forward and backward components the reduction and oxidation currents were equal and occurred at the same potential value, Figure 6B.

4 Discussion

The isoflavones genistein and daidzein have a hydroxyl group in the B-ring (Scheme 1A and C), which is oxidised at a low potential, Peak 1_a (Figure 3A and C and Table 2) whereas biochanin A lacks the hydroxyl group in the B-ring, Scheme 1B, and the anodic Peak 1_a is absent (Figure 3B and Table 2). The oxidation of this hydroxyl group in B-ring led to the formation of one oxidation product, Peak 5_a, as confirmed by the cyclic voltammograms (Figure 1A), where the scan direction was reversed immediately after Peak 1_a. This oxidation product undergoes reversible redox reactions (Figure 6B).

Genistein and biochanin A (Scheme 1A and B) have two hydroxyl group attached to ring A, forming a resorcinol structure. Each of these hydroxyl groups is oxidised at different potential values, Peak 2_a and 3_a (Figure 3A

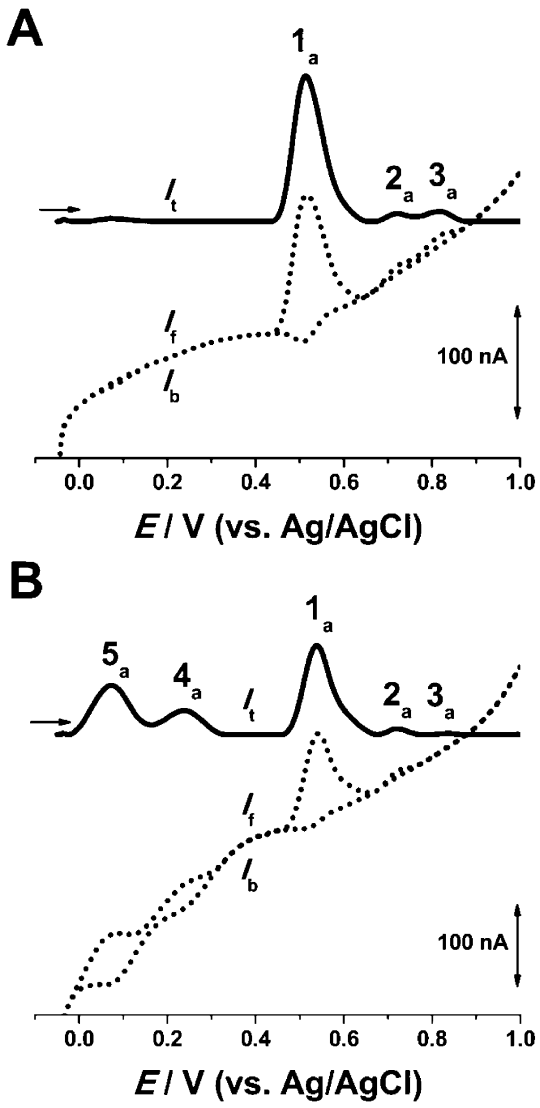


Fig. 6. SW voltammograms baseline-corrected in 5 μM genistein in pH 7.0: A) first and B) second scans; I_t, I_f and I_b – total, forward and backward currents. $f=25$ Hz, $\Delta E_s=2$ mV for $v=50$ mV s^{-1}.

and B and Table 2). Voltammograms of daidzein (Figure 3C) with only a hydroxyl group in A-ring (Scheme 1C) enabled the identification of the oxidation process at Peak 2_a. The oxidation of the hydroxyl group in ring A of daidzein led to the formation of only one oxidation product Peak 5_a (Figure 3C and Table 2).

The oxidation of the hydroxyl groups in the A-ring and B-ring is similar to the oxidation of a phenol moiety, which initially involves the formation of a radical cation which reacts with water resulting in *ortho*- and *para*-quinones [26,27,31]. The electroactive oxidation products of the hydroxyl groups in B-ring and/or A-ring, correspond to Peaks 5_a–5_c oxidation of an *ortho*-quinone, and Peaks 4_a–4_c to the oxidation of a *para*-quinone.

The absence of Peaks 4_a–4_c in daidzein (Scheme 1C) indicates that the *para*-quinone type oxidation product is correlated with the electrochemical oxidation of the hydroxyl group on the A-ring at position 5, present in genistein and biochanin A (Scheme 1A and B) and the Peaks 5_a–5_c are related with the oxidation of the hydroxyl groups at position 4′ in the B-ring and 7 in the A-ring.

The occurrence of Peak 6_a in biochanin A solutions (Figures 5A and B) is related to the oxidation of a specie formed by a chemical reaction between the initial radical cation with a biochanin A molecule through the methoxyl group on the B-ring. In fact, biochanin A is the only molecule that contains a methoxyl group on the B-ring. The absence of Peak 6_a on the voltammograms recorded in solutions of genistein and daidzein is explained by the steric effects that impede a similar reaction.

5 Conclusions

The electrochemical oxidation mechanisms of isoflavones genistein and biochanin A, compounds with anticancer properties and inhibitors of protein tyrosine kinases, were investigated at a glassy carbon electrode by cyclic, differential pulse and square wave voltammetry. It has been shown that in acid, neutral and mild alkaline electrolytes, each hydroxyl group of genistein undergo irreversible, pH dependent oxidation with the transfer of one electron and one proton. For strong alkaline media, two, pH independent charge transfer reactions were observed. The oxidation of genistein involves the formation of two oxidation products that undergo two electrons and two protons reversible redox reactions.

The electrochemical behaviour of biochanin A and daidzein, two genistein chemical analogues, was also studied in order to identify genistein electroactive centres. Both compounds undergo two consecutive, irreversible and pH dependent redox reactions, the peak potential being influenced by the position of the hydroxyl groups in their structures.

An oxidation mechanism has been discussed.

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References

[1] M. Comalada, J. Xaus, J. Gálvez, in *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (Eds: Watson, Preedy), Academic Press, San Diego, **2013**, ch. 42, pp. 555–579.

[2] F. Menaa, S. L. Badole, B. Menaa, A. Menaa, S. L. Bodhankar, in *Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases* (Eds: R. R. Watson, V.

Preedy), Academic Press, San Diego, **2013**, ch. 32, pp. 421 – 430.
[3] C. Ruefer, S. E. Kulling, *J. Agric. Food Chem.* **2006**, *54*, 2926.
[4] I. C. W. Arts, P. C. H. Hollman, *Am. J. Clin. Nutr.* **2005**, *81*, 317S.
[5] M. Kampa, A. P. Nifli, G. Notas, E. Castanas, *Rev. Physiol. Biochem. Pharmacol.* **2007**, *159*, 79.
[6] J. J. Peterson, J. T. Dwyer, P. F. Jacques, M. L. McCullough, *Nutr. Rev.* **2012**, *70*, 491.
[7] K. Taku, M. K. Melby, N. Nishi, T. Omori, M. S. Kurzer, *Maturitas* **2011**, *70*, 333.
[8] D. J. Lamport, L. Dye, J. D. Wightman, C. L. Lawton, *Nutrition and Aging* **2012**, *1*, 5.
[9] T. G. Peterson, G. P. Ji, M. Kirk, L. Coward, C. N. Falany, S. Barnes, *Am. J. Clin. Nutr.* **1998**, *68*, 1505S.
[10] E. Szliszka, Z. P. Czuba, A. Mertas, A. Paradysz, W. Krol, *Urol Oncol*. **2011**, doi:10.1016/j.urolonc.2011.01.019, in press.
[11] K. Polkowski, A. P. Mazurek, *Acta Pol. Pharm.* **2000**, *52*, 135.
[12] S. A. Khan, R. T. Chatterton, N. Michel, M. Bryk, O. Lee, D. Ivancic, R. Heinz, C. M. Zalles, I. B. Helenowski, B. D. Jovanovic, A. A. Franke, M. C. Bosland, J. Wang, N. M. Hansen, K. P. Bethke, A. Dew, M. Coomes, R. C. Bergan, *Cancer Prev. Res. (Phila.)* **2012**, *5*, 309.
[13] S. J. Nechuta, B. J. Caan, W. Y. Chen, W. Lu, Z. Chen, M. L. Kwan, S. W. Flatt, Y. Zheng, W. Zheng, J. P. Pierce, X. O. Shu, *Am. J. Clin. Nutr.* **2012**, *96*, 123.
[14] T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami, *J. Biol. Chem.* **1987**, *262*, 5592.
[15] S. K. Manna, *Biochem. Pharmacol.* **2012**, *83*, 1383 – 1392.
[16] S. Banerjee, Y. Li, Z. Wang, F. H. Sarkar, *Cancer Lett.* **2008**, *269*, 226.
[17] J. Zielonka, J. Bicki, G. Grynkiewicz, *Free Rad. Biol. Med.* **2003**, *35*, 958.
[18] J. Zhang, F. Du, B. Peng, R. Lu, H. Gao, Z. Zhou, *J. Mol. Struct.- Theochem.* **2010**, *955*, 1.
[19] M. A. Samra, V. S. Chedea, A. Economou, A. Calokerinos, P. Kefalas, *Food Chem.* **2011**, *125*, 622
[20] I. P. G. Fernandes, S. C. B. Oliveira, M. Ghalkhani, S. Shahrokhian, A. M. Oliveira-Brett, *Electroanalysis* **2012**, *24*, 618.
[21] J. Koyama, I. Morita, N. Kobayashi, T. Konoshima, M. Takasaki, T. Osakai, H. Tokuda, *Cancer Lett.* **2008**, *263*, 61.
[22] L. Wu, J. B. Laughlin Jr., H. D. Dewald, *Electroanalysis* **1997**, *9*, 796.
[23] X. Zhang, J. Zheng, H. Gao, *Anal. Lett.* **2001**, *34*, 1901.
[24] B. Klejdus, J. Vacek, V. Adama, J. Zehnálek, R. Kizek, L. Trnková, V. Kubán, *J. Chromatogr. B* **2004**, *806*, 101.
[25] R. Mikelová, P. Hodek, P. Hanustiak, V. Adam, S. Krizkova, L. Havel, M. Stiborova, A. Horna, M. Beklova, L. Trnkova, R. Kizekc, *Acta Chim. Slov.* **2007**, *54*, 92.
[26] V. C. Diculescu, H. E. Satana, E. de Souza Gil, A. M. Oliveira Brett, *Electroanal*ysis **2012**, *24*, 1019.
[27] M. A. N. Manaia, V. C. Diculescu, E. D.S. Gil, Ana M. Oliveira-Brett, *J. Electroanal. Chem.* **2012**, *682*, 83.
[28] C. M. A. Brett, A. M. Oliveira Brett, *Electrochemistry: Principles, Methods and Applications*, Oxford Science University Publications, Oxford, UK **1993**.
[29] A. J. Bard, L. R. Faulkner, *Electrochemical Methods. Fundamentals and Applications*, 2nd ed., Wiley, New York **2001**.
[30] O. M. Popa, V. C. Diculescu, *J. Electroanal. Chem.* **2013**, *689*, 216.
[31] T. A. Enache, A. M. Oliveira Brett, *J. Electroanal. Chem.* **2011**, *655*, 9.

