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Eletrochemical characterization of Scopoletin, a 7-hydroxy-6-methoxy-coumarin

Karla Carneiro de Siqueira Leite[1], *Lívia M. F. C. Torres*[2], *Luane Ferreira Garcia*[1], *Stefani Garcia Rezende*[1], *Jeronimo Raimundo de Oliveira-Neto*[1], *Flávio Marques Lopes*[1], *Telma Alves Garcia* [1], *Rodrigo M. Verly*[2], *Wallans T. P. dos Santos*[3], *Eric de Souza Gil*[1,*]

[1] Faculdade de Farmácia, Universidade Federal de Goiás, 74605-220, Goiânia, Goiás, Brasil
[2]Departamento de Química, [3]Departamento de Farmácia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39100-000, Diamantina – MG, Brasil
[*]E-mail: ericsgil@gmail.com

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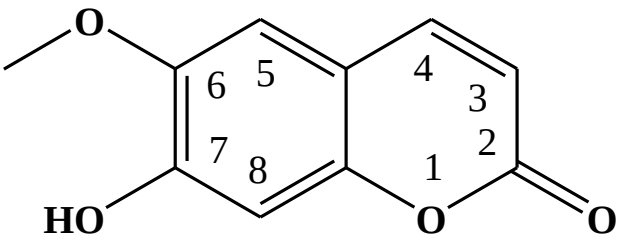
The coumarins are an important class of phytochemicals with wide range of biological activities. The 7-hydroxy-6-methoxycoumarin, scopoletin is a biomarker widespread in plant kingdom, especially in the phytotherapic and nutraceutical ones. A considerable number of patents and scientific papers concerning its amazing pharmacological properties highlights the relevance of scopoletin. How your benzo-α-pyrone skeleton, resembling the flavonoid antioxidants, the electrochemical characterization of scopoletin can be a useful tool to understand the behavior between chemical and biological properties. Therefore, the aim of this work was to develop the electrochemical characterization of scopoletin at glassy carbon electrode, in a wide range of electrolyte conditions by means of cyclic, differential pulse and square wave voltammetry. Thus, it was found that akin to what would be expected for phenolic compounds, the 7-hydroxy group undergoes electrochemical oxidation at Ep1a ~ 0.7 V, meanwhile it leads to different oxidation products that showed to be oxidized at lower potentials. An oxidation mechanism was proposed, considering the results herein obtained.

Keywords: Coumarins, Cinnamic acid derivatives, Poliphenolic compounds, Noni, Nutraceuticals, Voltammetry.

1. INTRODUCTION

Scopoletin, is a hydroxylatedcoumarin, 7-hydroxy-6-methoxy-2H-1-benzopyran-2-one, (Scheme 1) widespread in plant kingdom. It is a biologically active constituent of many species of plants used in the traditional medicine and its importance has been highlighted due the increasing global interest on the nutraceutical properties of Noni [1-9].



Scheme 1 – Chemical structure of scopoletin.

It was first isolated by Eykman in 1884 from the rhizome of *Scopolia japonica,* whose name is derived [1]. However, due its occurrence in different plants, i.e. *Atropa belladonna* [1], *Althaea officinalis* [2], *Morindacitrifolia* [3], *Gelsemiumsempervirens* [4], *Tiliacordata* [5], *Malvasilvestris*[6] and, *Menthaspicata* [7], scopoletin has also been named as gelseminic acid, chrysatropic acid, 7-hydroxy-6-methoxycoumarin and β-methylaesculetin [1].

The pharmacological benefits of scopoletin and the plants that contain it are diverse, hence including antifungal [8-10], antitumoural [4,5], anti-inflammatory [2,3], neurological [11-13], radical scavenging [14], hepatoprotective [15-18] activities.

In fact, the main uses of scopoletin and related phytotherapics are attributed to their anti-inflammatory properties [2-3]. Although, there is not a unique and specific proposal for its mechanism of action, the inhibition of inflammatory cytokines seems to be the most convergent [4, 19, 20]. Yet, this proposal is consistent with scopoletin's potential use as therapeutic agent for angiogenesis-related diseases, i.e. rheumatoid arthritis, diabetic retinopathy and cancer [3,19,20]. Accordingly, the wide pharmacological profile of Noni (*Morindacitrifolia*) is akin to scopoletin, its main biomarker [3,21-24]. Thus, as a consequence of the impressiveness therapeutic success of Noni products, this tropical fruit has reached the global market [24].

In turn, owing its relevance, scopoletin has not only been isolated from different vegetal sources [13,14,25], but also some attempts to its synthesis were proposed [26,27]. Furthermore, the importance of scopoletin is also expressed by a considerable number of deposited patents [25,27].

Nevertheless, the greater the relevance of a chemical compound, the higher the need for its analysis. Indeed, the development of methods for quality control of phytopharmaceuticals is always mandatory and generally complicated [28].

The analysis of the phytopharmaceutical, scopoletin has been carried out mainly by chromatographic methods [24, 28-30]. Though these methods are very suitable and precise to phytotherapics analysis, they are time and reagent consuming [28-30]. In this context, the electroanalytical techniques have showed to be very useful on the analysis of coumarins [31-33]. Nevertheless, to best of our knowledge no electrochemical study of scopoletin has been done. This study is very useful not only from the chemical characterization point of view, but also to understand some biological properties.

Therefore, the aim of this work is carring out the electrochemical characterization of scopoletin, at glassy carbon electrode in different supporting electrolyte conditions by means of cyclic voltammetry (CV), Differential pulse voltammetry (DPV) and Square Wave Voltammetry (SWV).

2. MATERIAL AND METHODS

2.1 Materials and Reagents

Scopoletin was purchased from Sigma Aldrich (Saint Louis, MO, USA) and used without further purification. Stock solutions were prepared in ethanol-deionised water (50:50, v/v) and stored at 5 ºC. Solutions of different concentrations of scopoletin were prepared by dilution of the appropriate quantity in supporting electrolyte.

All supporting electrolyte solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1}) [34].

2.2 Apparatus

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Eco-Chemie, Utrecht, 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 = 1.5 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 Crisonmicro pH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 ºC) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV s-1. For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 50 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 100 mV s^{-1} were used.

The GCE was polished using alumina 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 very reproducible experimental results.

2.3 Acquisition and Presentation of Voltammetric Data

All the voltammograms presented were background-subtracted and baseline-corrected using the moving average application with a step window of 5 mV included in GPES version 4.9 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 (<10%) when compared to a case of 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 AND DISCUSSION

3.1 Cyclic Voltammetry

Coumarins are derivatives of cinnamic acid with a benzo-α-pyrone skeleton, which resembles the flavonoidic rings A and C [35]. Therefore, the hydroxy groups on benzo ring may have a similar behaviour that one expected to widely studied flavonoids. The coumarin, scopoletin has an oxidizable hydroxyl group at position 7, meanwhile the methoxy group at the position 6 can drive the secondary electrode reactions [34, 35].

Cyclic voltammograms (CVs) in 75 μM scopoletin in pH = 4.0 0.1 M acetate buffer recorded from + 0.00 to +1.10 V, Fig. 1, showed a single anodic peak, 1a, at E_{p1a} = + 0.69 V. Reversing the scan direction, on the negative-going scan of the first CV, two close cathodic peaks: peak 1c, at E_{p1c} = 0.53 V, and peak 2c, at E_{p2c} = + 0.41 V, occurred, due to the reduction of scopoletin oxidation products. Successive CVs up to the third scan obtained in the same solution showed two new anodic peaks: peak 2a, at E_{p2a} = + 0.47 V, and peak 3a, E_{p3a} = + 0.61 V, whilst on the reverse scan the cathodic peaks, 1c and 2c showed to become less closer, Fig. 1.

Hereafter, on continuous CVs scans, there are loss of peaks resolution, being observed expressive fall of current levels and displacement of potentials to a less reversible profile, Fig 1, Table 2. Furthermore, the peak 3a, which was clearly identified only in the third scan, showed to merge with peak 1a on the following scans, Fig. 1.

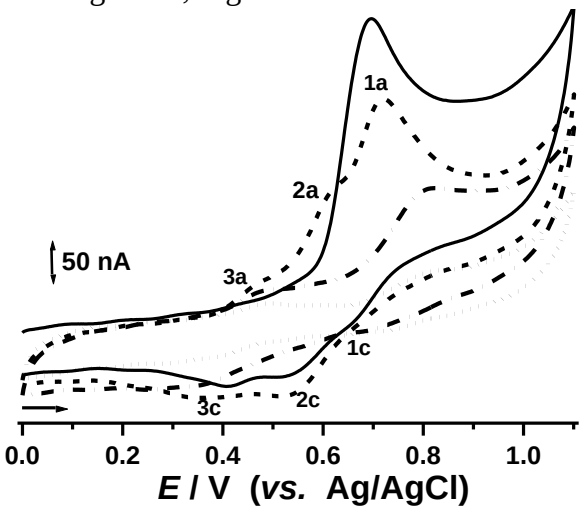


Figure 1.Cyclic voltammograms in pH = 4.0 0.1 M acetate buffer of 75 μM scopoletin; (▬) first,(- - -) third, (-•-) tenth and (•••) twentieth scans at v = 50 mV s^{-1}, scan range from 0 to 1.10 V.

The complex CV profile of scopoletin clearly indicates that the electrochemical mechanism of oxidation involves chemical coupled processes. Meanwhile, the shifting and decrease of scopoletin

anodic peaks upon successive CVs is consistent with electropolymerization leading to expressive changings of electrode surface. Indeed, the electrochemical production of diffusion less film has been also described on literature [35-38].

Table 2. Epa values obtained in 1st and 2nd CV scans for natural dyes at CGE in 0.2 M Phosphate buffer, pH 6.0 at scan rate of 50 mV.s^{-1}.

Peak / Scan	E_{p1a} (mV)	I_{p1a} (nA)	E_{p2a} (mV)	I_{p2a} (nA)	E_{p3a} (mV)	I_{p3a} (nA)	E_{p1c}(mV)	I_{p1c}(nA)	E_{p2c}(mV)	I_{p2c}(nA)
1st	696	340	-	-	-	-	526	- 49	409	- 61
3rd	718	252	465	50	609	146	528	- 73	345	- 70
10th	801	157	488	45	-	-	349	- 60	249	- 63
20th	820	66	488	30	-	-	393	- 27	288	- 35

The effect of scan rate on the main anodic peak, 1a of scopoletin was also evaluated. Increasing the scan rate the potentials of all peaks were shifted to more positive values. In turn, the main anodic peak current, I_{p1a} is directly proportional to square root of scan rate ($\nu^{1/2}$) rather than to the scan rate (ν), thus indicating that the oxidation of scopoletin is a diffusion controlled process, Fig. 2.

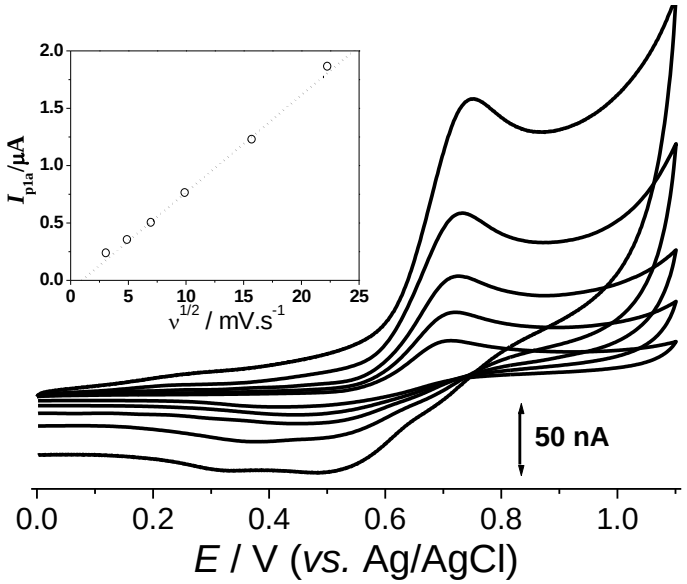


Figure 2. CVs scans obtained for 75 µM Scopoletin in pH 4.0 0.1 acetate buffer obtained at different scan rates: 10, 25, 50, 100 and 250 mV.s^{-1}. Inset: I_{p1a} *vs* $\nu^{1/2}$plot.

Furthermore, the higher ΔEp values, as well the $I_{pa}/I_{pc} \neq 1$ for all peaks and at all sweep rates clearly show the commitment of the quasi-reversible behavior.

On the other hand, although the peak current function, $I_{p1a}/\nu^{1/2}$ remains almost constant, the ratio I_{pa}/I_{pc} is higher than unit and increases with sweep rate, indicating the presence of coupled

chemical (EC) reactions [35-38]. Indeed, the complex electrochemical behavior, showed on Fig. 1 and Table 2 is also indicative of coupled reactions.

However, some peaks are not so well defined in cyclic voltammetry. Therefore, in order to better evaluate the each peak, the DPV was carried out.

3.2 Differential Pulse Voltammetry

The pH effect on the electrochemical oxidation of scopoletin was investigated by means of DPV, which allows lower detection limits and a better visualisation of all redox processes.

Therefore, in order to achieve the influence of pH on the electrode reactions, DP voltammograms were successively recorded without cleaning the electrode surface in acidic, pH = 2.0; neutral, pH = 7.0; and alkaline, pH = 9.0 medium, Fig. 3.

The first DP voltammograms recorded in 25 μM scopoletin showed one single anodic peak, 1a, which showed to diminish intensely on repeated scans, Fig. 3. As mentioned before, the peak current fall is perhaps a consequence of film formation leading to the reduction of the available electrode surface area [35].

Furthermore, as demonstrated in CVs for repeated scans (Fig. 1), the appearance of peaks 2a and 3a at lower potentials were also observed in subsequent DP voltammograms in acid and neutral pH, Fig. 3A and 3B. By contrast, when the experiment was carried out in alkaline medium, these peaks, 2a and 3a were not observed, Fig. 3C, whilst a fourth peak 4a, appears in pH 2.0, Fig. 3A.

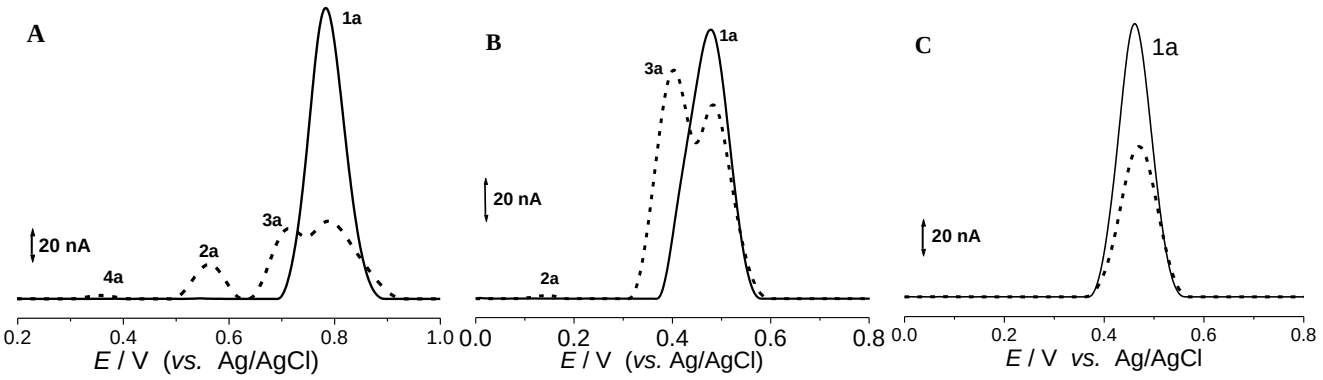


Figure 3. DP voltammograms base-line corrected recorded for 25 μM scopoletinin: pH 2.0 (A), pH 7.0 (B) and pH 9.0 (C); (▬) first and (▪▪▪) second scans. Scan rate of 10 mV s^{-1}, pulse amplitude of 50 mV.

Therefore, regardless the results showed in Fig. 3, it can be inferred that the electrode reactions pathways are greatly driven by pH conditions. Thus, the DPV profile suggests that subsequent coupled chemical reaction may be prevented under alkaline medium, meanwhile in stronger acid medium they may be favored.

The pH dependence of the main anodic peak, 1a was evaluated on a wide range (pH 2.0 to 12,0). The peak potentials shifted to lower values with pH increase in a liner manner up to pH 7.5 and then become pH independent, Fig. 4

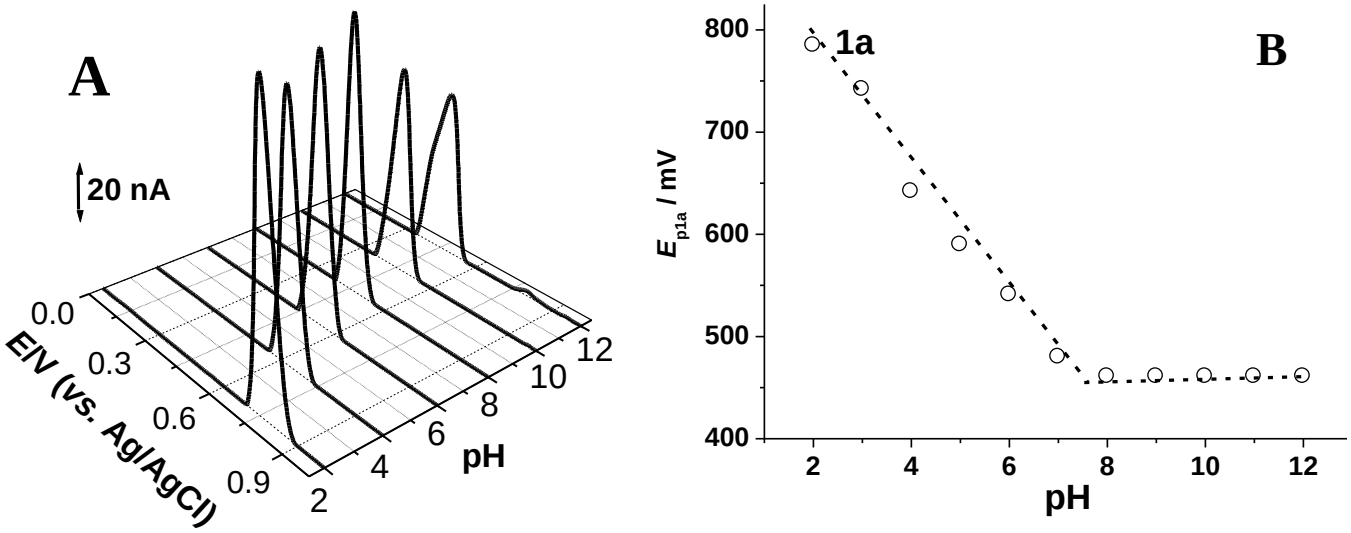


Figure 4. 3D plot of first scan DP voltammograms base-line corrected (A) and corresponding E_{p1a} *vs* pH plot (B) obtained for 25 μM scopoletin. Scan rate of 10 mV s^{-1}, pulse amplitude of 50 mV.

Moreover, the slope of ~ 60 mV per pH unit in the Epa *vs*. pH plots and the width at half-height of the peak, 1a (~ 100 mV) indicate an oxidation reaction with the transfer of one electron and one proton [34,35]. Herein, these accounts are in complete agreement with the electrochemical oxidation of the 7-hydrox group, being in agreement with phenol oxidation mechanisms [35]. In turn, considering the improbable presence of other eletroactive groups for the experimental conditions herein used, it can be inferred that the peaks 2a, 3a and 4a are related to phenol oxidation products [35, 37].

In order to achieve a better understanding of each redox peak, the SWV experiments were also carried out.

3.3 Square Wave Voltammetry

The electrochemical behaviour of scopoletin was also evaluated by SW voltammetry. In this technique the current is sampled in both positive and negative-going pulses, hence the oxidation and reduction peaks of the electroactive compound at the electrode surface can be obtained simultaneously and the reversibility of the electron transfer reaction can be better checked by plotting the forward and backward components of the total current, enabling lower detection limits than CV. On the other hand, the fast sweep rate leads to lower adsorption products, which usually improves the reproducibility in quantitative determinations.

Nevertheless, the SWV experiments were employed with the main purpose of ascertaining qualitative characteristics of the main oxidation product peaks, 2a and 3a, thus improving the overall electrochemical behaviour of scopoletin.

Therefore, in order to achieve this goal, this technique was performed in a wide pH range not only for cleaned electrode surface, but also immediately after DP experiments. Furthermore, due to the high amount of adsorbed species on the electrode surface, this procedure showed to be more sensitive on the evaluation of the reversibility of each redox process.

Fig. 5 shows the first scan SW voltammograms obtained when GC electrode was cleaned just prior to use. Under this condition, a unique large anodic process can be observed, whereas on the backward component show the overall process is not completely irreversible, Fig 5A.

In turn, the SW voltammograms obtained at GC electrode without any cleaning procedure and immediately after a first scan DP experiment (all conditions as mentioned in Fig. 3), clarify the reversibility of peaks 2a and 3a, thus suggesting the possibility of formation of ortho and paraquinone products. On the other hand, the small intensity of these peaks, as well the peak current fall on successive scans is coincident to concomitant electropolymerization reactions, thus leading to insulator film on the electrode surface [35-38].

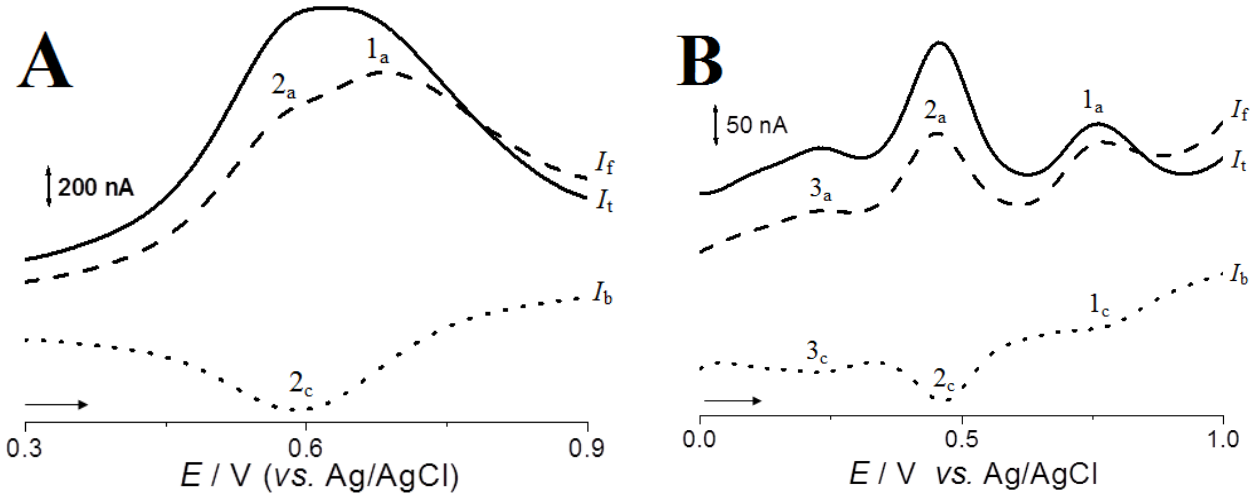
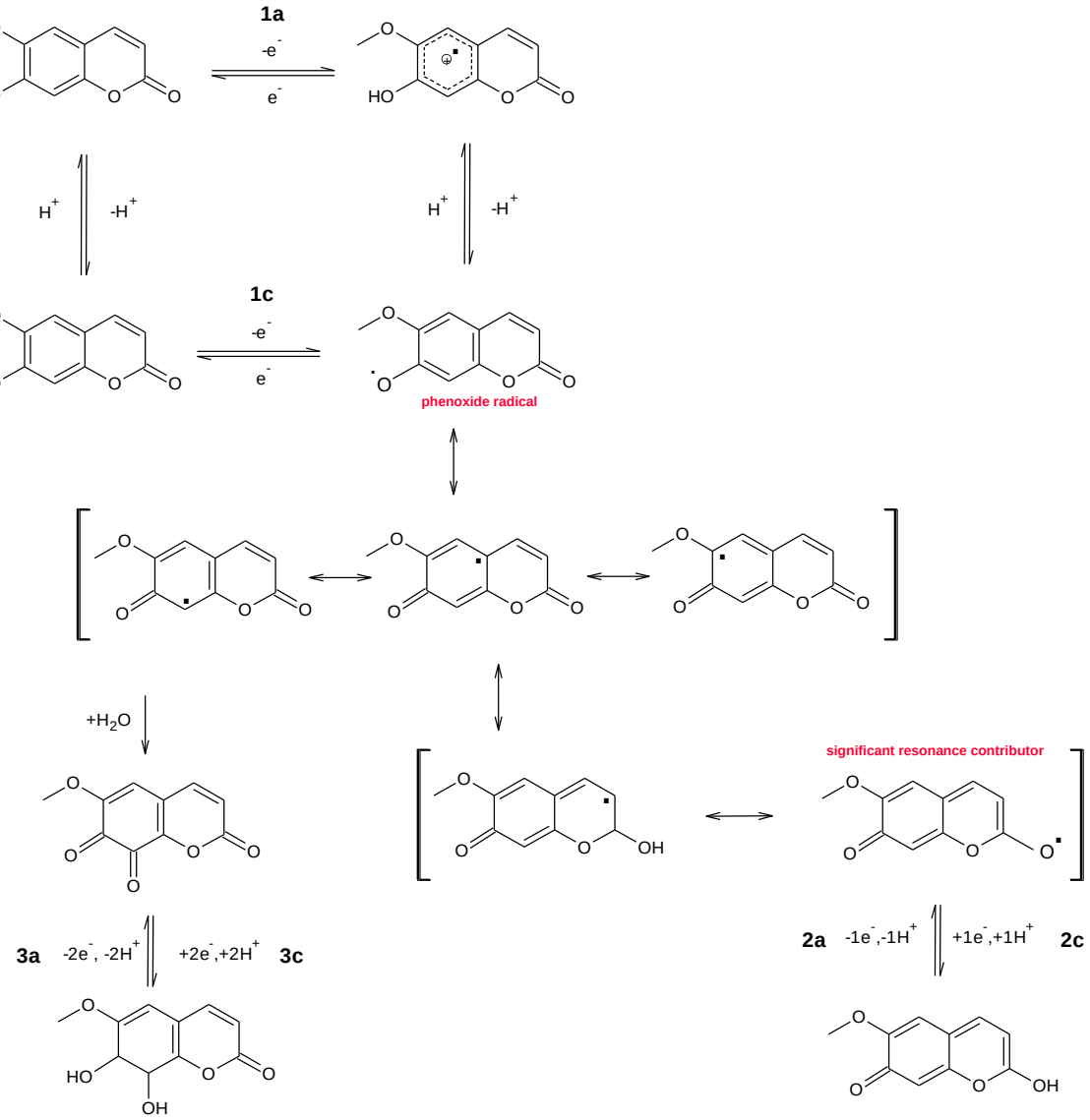


Figure 5. SW voltammograms of 75 μM scopoletin in pH = 3.0: (A) first and (B) fifth scan; f = 50 Hz, ΔEs = 2 mV, v_{eff} = 100 mV s^{-1}; I_t - total, I_f - forward and I_b – backward.

3.4 Oxidation Mechanism

Analysis of the isolated compounds such as 7-hydroxy-6-methoxycoumarin allows the electrochemical characterization and also their correlation with the proposed mechanism of oxidation. The course of the electrooxidation of derivative phenolic study is remarkably complex, as indicated in Eq. 1.



Equation 1. Oxidation mechanism of 7-hydroxy-6-methoxycoumarin

The many species involved are related to one another by a series of proton and electron transfers that may occur as the result of bimolecular interactions as well as by primary electrode processes [37-39]. Like observed in DPV, where the slope of ~ 60mV per pH unit in the E_{pa} *vs.* pH plots and the width at half-height of the peak, 1a (~100mV) Fig. 4, the 7-hydroxy-6-methoxycoumarin is oxidized in an one-electron and one proton step, to a derivative phenoxide radical and then it seeks an electron to form a phenoxide radical (Eq. 1,process 1a). Delocalization of its unpaired electron strongly stabilizes phenoxide radical that is shared by the oxygen and benzene ring, attributing highest spin density to the carbons that are ortho and para position. The hydrolysis at a high potential at ortho-position followed by irreversible oxidation leads to the formation of derivative diol structure (process 3c) and, as mentioned in Fig. 3, the reversibility of peaks 2a ande 3a, also suggests the possilitity of formation of ortho and para-quinone products. On the other hand, there is an extended conjugation in the para-position suggesting a greater resonance contributor to the phenoxide radical.

Consequently, a reduction process and proton step result in obtaining of another derivative phenol (Eq. 1, process 2a).

The other product could be obtained via phenol oxidations involving phenoxy radicals from simple ortho- or para- substituted as 7-hydroxy-6-methoxycoumarin. Suitably substituted phenoxy radicals with sterically bulky ortho-substituents R and non-bulky para-substituents R/ (Me, Et, Bu) have been found to exist in equilibrium with their corresponding para-quinol ether derivatives (VII), as in Eq.1. The results are more complex when R' is bulkier [40, 41].

The complex CV profile of scopoletin clearly indicates that electrooxidation of derivative phenol 7-hydroxy-6-methoxycoumarin, could result at oxidative C-C coupling of derivative phenoxy radicals and could lead to a symmetrical biphenyl derivatives as observed in anodic oxidation represented in Eq. 1 [35,37].

4. CONCLUSIONS

Coumarins, are an important class of natural compounds with remarkable biological activities, i.e. anti-inflammatory, antioxidant and antitumoural activities. Belonging to this important class of phytochemicals, scopoletin has shown increased chemopreventive, hepatic and neuroprotective properties. Such pharmacological properties show to be according to the therapeutical uses of the main phytotherapics that contain scopoletin as biomarker. Nevertheless, owing to the presence of phenolic and methoxy groups, scopoletin is electroactive in nature, and also exhibits interesting redox properties. Thus, the electrochemical behaviour of scopoletin was investigated considering its structural pattern. The hydroxyl group in the benzo ring showed to undergo electrochemical potential on the expected potential for phenolic compounds. Therefore, this process showed to be a one-electron, one-proton irreversible pH-dependent process. The electrochemical oxidation is coupled to chemical reactions leading to different oxidation products.

The occurrence of different oxidation products involving the formation of para- and ortho-quinones “like products” that undergo reversible redox reaction was observed and their electrochemical behaviour characterised. It has been observed that the presence of methoxy group in ortho position drives the pathways mechanisms of oxidation, favouring the formation of a less convoluted polymeric film. Nevertheless, as the film grows on repeated scans, the electrode passivation is highlighted.

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References

1. C. W. Moore, *J. Chem. Soc*. 99 (1911) 1043-1048.
2. S. M. A. Shah, N. Akhtar, M. Akram, P. A. Shah, T. Saeed, K. Ahmed, H. M. Asif, *J. Med. Plants Res*.5 (2011) 5662-5666.

3. S. Mahattanadul, W. Ridtitid, S. Nima, N. Phdoongsombut, P. Ratanasuwon, S. Kasiwong, *J. Ethnopharmacol*. 134 (2011) 243-250.
4. A.R. Khuda-Bukhsh, S.S. Bhattacharyya, P. Saili, N. Boujedaini. *J. Chin. integrative Med*. 8 (2010) 853-62.
5. M.L.B. Arcos, G. Cremaschi, S. Werner, J. Coussio, G. Ferraro, C.Anesini, *Phytoth. Res.* 20 (2006) 34-40.
6. M. Della-Greca, F. Cutillo, B. D'Abrosca, A. Fiorentino, S. Pacifico, A. Zarrelli, *Nat. Prod. Comm.* 4 (2009) 893-89.
7. M. Adam, P. Dobias, A. Eisner, K. Ventura, *J. Separat*. *Sc.* 32 (2009) 288-294.
8. J. Lee, N.H. Kim, J.W. Nam, Y.M. Lee, D.S. Jang, Y.S. Kim, S. H. Nam, E.K. Seo, M. S. Yang, J. S. Kim, *Arch. Pharm. Res*. 33(2010) 1317-1323.
9. M.C. Carpinella, C.G. Ferrayoli, S.M. Palacios, *J. Agric. Food Chem.* 53 (2005) 2922–2927.
10. T. Valle, J.L. Lopez, J.M. Hernandez, P. Corchete, *Plant Sc.* 125 (1997) 97-101.
11. J.C. Capra, M. P. Cunha, D. G. Machado, A.D.E. Zomkowski, B.G. Mendes, A.R. S. Santos, M.G. Pizzolatti, A.L.S. Rodrigues, *Eur. J. Pharmacol*. 643 (2010) 232-238.
12. A. Hornick, A. Lieb, N.P. Vo, J.M. Rollinger, H. Stuppner, H. Prast, *Neurosc.*, 197 (2011) 280-292.
13. N. Mishra, A. Oraon, A. Dev, V. Jayaprakash, A. Basu, A. K. Pattnaik, S. N. Tripapthi, M. Akhtar, S. Ahmad, S. Swaroop, M. Basu, *J. Ethnopharmacol.* 128 (2010) 533-6.
14. P.T. Thuong, T.M. Hung, T.M. Ngoc, D.T. Ha, B.S. Min, S.J. Kwack, T.S. Kang, J.S. Choi, K. Bae, *Phytoth. Res*. 24 (2010) 101-106.
15. J.R. Noh, Y.H. Kim, G.T. Gang, Gil-Tae, J. H. Hwang, H.S. Lee, S.Y. Ly, W.K. Oh, K.S. Song, C.H. Lee, *Food Chem. Toxicol*. 49 (2011) 1537-1543.
16. O.S. Kwon, J.S. Choi, M.N. Islam, Y.S. Kim, H.P. Kim, *Arch. Pharm. Res*. 34 (2011) 1561-1569.
17. M.A. Mohamed, M.S.A. Marzouk, F.A. Moharram, M. M.El-Sayed, A.R. Baiuomy, *Phytochem.* 66 (2005) 2780-2786.
18. S. Y. Kang, S. H. Sung, J. H. Park, Y. C. Kim, *Arch. Pharm. Res.* 21 (1998) 718-722.
19. P. D. Moon, B. H.Lee, H. J.Jeong, H. J.An, S. J. Park, H. R.Kim, S. G. Ko, J. Y. Um, S. H. Hong, H. M.Kim, *Eur. J. Pharmacol.* 555 (2007) 218-225.
20. R. Pan, X. H. Gao, D. Lu, X. X. Xu, Y. F. Feng, Y. Dai, *Int. Immunopharmacol*. 11 (2011) 2007-2016.
21. V. Samoylenko, J. P. Zhao, D. C. Dunbar, I. A. Khan, J. W. Rushing, I. Muhammad, *J. Agric. Food Chem.* 54 (2006) 6398-6402.
22. H. K. Beh, Z. Ismail, M. Z. Asmawi, W. S. Loh, H. K. Fun, *ActaCrystallograph*. 66 (2010) O2138-U2257.
23. S. Prapaitrakool, I. Arunporn, *J. Med. Assoc. Thailand*. 93 (2010) S204-9.
24. S. X. Deng, B. J. West, C. J. Jensen, *Food Chem*. 122 (2010) 267-270.
25. R. S. Bhakuni, M. M. Gupta, S. K. Gupta, D. C. Jain, A. P. Kahol, S. Kumar, N. Pant, S. Tandon, A. Tiwari, R. K. Verma, Improved process for isolation of compound scopoletin useful as nitric oxide synthesis inhibitor from *Artemisia annua*, Patent IN191626-B (2009).
26. D. G. Crosby, *J. Org. Chem.*, 26 (1961) 1215–1217.
27. Z. Bian, S. Hu, H. Liang, J.Li, F. Pan, Y. Peng, Y. Zhang, L. Zou, Use of scopoletin for preparing medicament for prevention or treatment of tumor e.g. colon cancer, Patent CN101401808-A (2009).
28. N. S. Kapadia, N. S. Acharya, S. A. Acharya, M. B. Shah, *J. Planar Chromatogr. Modern TLC.* 19 (2006) 195-199.
29. F. Li, Q. Liu, W. Cai, X. Shao, *J. Chromatog*. 69 (2009) 743-748.
30. Y. C. Zhang, H. Y. Xu, X. M. Chen, C. Chen, H. J. Wang, F. Y. Meng, H. J. Yang, L. Q. Huang, *J. Pharm. Biomed. Anal.*, 56 (2011) 497-504.
31. X. R. Hu, J. B. He, Y. Wang, Y. W. Zhu, J. J. Tian, *Electrochim. Acta,* 56 (2011) 2919-2925.
32. V. Pardo-Jimenez, C. Barrientos, J. A. Squella, P. A. Navarrete-Encina, L. J. Nunez-Vergara, *J. Electrochem. Soc.*, 58 (2011) F166-F172.

33. H. Salehzadeh, D. Nematollahi, M. Rafiee, *J. Electroanal. Chem*., 650 (2011) 226-232.
34. D.D. Perrin, B. Dempsey in: *Buffers for pH and Metal Ion Control*, Chapman and Hall Laboratory Manuals, London (1974).
35. T.A. Enache, A.M. Oliveira-Brett, *J. Electroanal. Chem*. 655 (2011) 9-16.
36. M. Ferreira, H. Varela, R.M. Torresi, G. Tremiliosi-Filho, *Electrochimica Acta*, 52 (2006) 434-442.
37. D.H. Evans, *Accounts of Chem. Res.* 10 (1977) 313- 319.
38. B. Speiser, A. Rieker, J. Electroanal. Chem., 102 (1979) 373-395.
39. E. S. Gil, R.O. Couto, *Rev. Bras. Farmacogn.*, 23:3 (2013) 542-558.
40. D. H. Evans, P. J. Jiminez, M. J. Kelly, *J. Electroanal. Chem.*163 (1984)145-157.
41. J. A. Richards, D. H. Evans., *J. Electroanal. Chem.* 81 (1977) 171-187.

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