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Electrochemical oxidation of fisetin: Studies related to its adsorption on glassy carbon electrodes

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ABSTRACT

The electrochemical oxidation of fisetin (FIS) on glassy carbon (GC) electrodes is studied in 15% dimethyl sulfoxide (DMSO) +85% pH 4.00 and 7.00 buffer solutions by cyclic (CV) and square-wave (SWV) voltammetries. The first oxidation peak of FIS showed an adsorption/diffusion mixed control, when studies were performed in solutions in the presence of FIS. Therefore, the FIS adsorption on the GC electrode surface could be inferred from cyclic voltammograms recorded in supporting electrolyte solutions, where a quasi-reversible redox couple was defined at both pH values. These findings show that both the reactant and the product of the electrode process are adsorbed on the electrode surface, being the surface redox couple slightly better defined at pH 4.00 than 7.00. The Frumkin adsorption isotherm was the best to describe the specific interaction of FIS with GC electrodes, using a fitting procedure of experimental fractional surface coverage (θ) vs. FIS bulk concentration (c_{FIS}^0). Moreover, we used the SWV to obtain a full characterization of the surface redox couple, applying the combination of the “quasi-reversible maximum” and the “splitting of the net voltammetric peak” methods. Values of (0.340 ± 0.003) V, 41 s^{-1} , and (0.49 ± 0.04) were determined for the formal potential, the formal rate constant, and the anodic transfer coefficient, respectively.

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1. Introduction

Flavonoids are a large group of substances widely distributed in the plant kingdom [1]. They have the general structure of a carbon skeleton, which contains two phenyl rings and a heterocyclic ring [2]. Flavonoids exhibit anti-cancer, anti-inflammatory, and antiviral properties, because of their antioxidant properties [3].

Fisetin (3,3',4',7-tetrahydroxyflavone, FIS, Fig. 1) is a flavonoid that has attracted significant attention from biological perspectives, considering its inhibitory action against HIV-1 proteinase, a virally encoded protein which is indispensable for maturation and processing of AIDS virus and a viable substrate for anti HIV therapy [4]. In addition, it has been shown that FIS may act in a similar way as α -tocopherol and could mimic its antioxidant activity in the membranes [5]. These effects have been mainly attributed to the antioxidant activity of flavonoids and their ability to inhibit enzymes involved in the production of oxygen reactive species [1]. Although flavonoids have received much attention in recent years, their basic electrochemical properties are not thoroughly discussed in the literature and their electrochemical oxidation mechanisms remain poorly understood [6–11].

Flavonoids are polyphenolic compounds which can be differentiated mainly on the basis of the structure of their heterocyclic ring (C ring in Fig. 1), and the amount and position of OH groups in their molecular structures. Hodnick et al. [6] studied the electrochemical behavior of several flavonoids, including FIS. They found that FIS is oxidized at low potentials on glassy carbon electrodes in dimethyl sulfoxide (DMSO) + pH 7.00 buffer solutions through a reversible process that involves two electrons and two protons.

In this way, the first oxidation peak of all flavonoids with a catechol group in B ring shows a behavior that can be described as a reversible or a quasi-reversible process, involving $2e^-$, $2H^+$ [6–10,12,13]. These results are in agreement with those obtained by Brett and Ghica [9] and Timbola et al. [11]. However, Timbola et al. [11] disagree with the interpretation given by other authors about the nature of oxidation peaks that flavonoids generally have at potentials more positive than the first oxidation peak, i.e. when the full range of anodic potential is studied.

On the other hand, the non-Faradaic preconcentration of FIS and other flavonoids on a carbon paste electrode and their subsequent determination by differential pulse voltammetry have been studied [14]. The determination of twelve flavonoids using adsorptive stripping voltammetry in a flow injection system was also reported [12]. A glassy carbon electrode chemically modified using FIS as modifier was employed to study the electrocatalytic oxidation of

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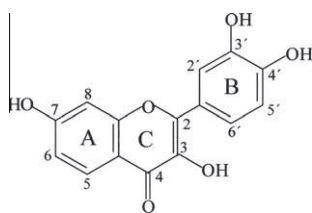


Fig. 1. Chemical structure of fisetin (3,3',4',7-tetrahydroxyflavone).

NADH and ascorbic acid [13]. Recently, Markovic et al. [15] discussed an experimental and theoretical study related to the antioxidant activity of FIS, in which cyclic voltammetry was used to obtain FIS redox potentials.

Moreover, it is known that different organic compounds have surface active properties on carbon electrodes [16,17]. The adsorption of these compounds can be studied by square wave voltammetry (SWV) as well as other techniques. It is also known that SWV is a particularly fast and sensitive technique, considering its ability to discriminate against capacitive currents [18,19]. In addition, SW voltammograms of surface redox processes are highly sensitive to the kinetics of the charge transfer [20]. They are characterized by both the so-called “quasi-reversible maximum”, where the ratio between the net peak current and the frequency is approximately a parabolic function of the kinetics parameter, κ , and the “splitting of the net voltammetric peak” with the SW amplitude at a given frequency [20,21]. According to the theory, the maxima of these parabolic functions and the splitting of net voltammetric peaks can be used to calculate the formal rate constant, the formal potential, and the anodic transfer coefficient of quasi-reversible redox couples immobilized on the electrode surface. The theory about the “quasi-reversible maximum” was initially developed assuming the absence of lateral interactions among the immobilized molecules [20]. However, as this condition is not satisfied by virtually any experimental system, new theoretical developments were proposed to include cases of surface redox reactions involving lateral interactions among adsorbed species [20,21].

Here, we study the first electrochemical oxidation peak of FIS on glassy carbon (GC) electrodes in 15% DMSO + 85% pH 4.00 and 7.00 buffer solutions by cyclic (CV) and SWV voltammetries. Cyclic voltammograms recorded in solutions in the presence of FIS showed an adsorption/diffusion mixed control. Therefore, the FIS surface redox couple was studied in 15% DMSO + 85% pH 4.00 buffer solution (supporting electrolyte solutions). We discuss the adsorption isotherm which best describes the experimental results. The formal rate constant (k_s), the anodic transfer coefficient ($1-\alpha$), and the formal potential (E_f^0) of the FIS quasi-reversible surface redox couple were determined from SWV, using a combination of the “quasi-reversible maximum” and the “splitting of the net voltammetric peak” methods. This methodology was previously used in our laboratory and demonstrated to be successful to perform a full characterization of altetroxin I adsorbed on carbon paste electrodes [16], as well as a full characterization of the cercosporin redox couple [22] and the ochratoxin A electro-oxidation product [23] adsorbed on GC electrodes. In addition, the application of the combination of the “quasi-reversible maximum” and the “splitting of the net voltammetric peak” methods was also recently used in our laboratory to study the thermodynamic and kinetics of the morin flavonoid adsorbed on GC electrodes [24].

The importance of the results obtained in the present study led us to carry out further studies in our laboratory, based on the application of anodic stripping voltammetry coupled to SWV to perform the electroanalytical determination of FIS in pharmaceutical formulations.

2. Experimental

2.1. Reagents

FIS (Sigma–Aldrich) was used as received. All other reagents were Merck, p.a. Stock solutions 1 mM of FIS were prepared in DMSO and kept in the refrigerator. Working solutions were prepared daily by adding aliquots of stock solutions to 15% DMSO + 85% pH 4.00 commercial buffer solutions (20 °C; citric acid + NaOH + HCl, reference number 109435) or pH 7.00 (20 °C; Na₂HPO₄ + KH₂PO₄, reference number 109439).

2.2. Apparatus and experimental measurements

The electrochemical experiments were performed using an Autolab PGSTAT 12 potentiostat, controlled by GPES 4.9 electrochemical software from Eco-Chemie, Utrecht, The Netherlands. Electrochemical measurements were carried out in a two-compartment Pyrex cell. The working electrode was a GC disk ($A = 0.070 \text{ cm}^2$) from Bioanalytical System, USA. Before each measurement, the working electrode was pretreated as follows: it was polished successively with wet alumina powder (0.3 and 0.05 μm , from Fischer), rinsed copiously with distilled water, and immersed in a distilled water ultrasonic bath for 2 min. The polished electrode was further activated electrochemically in 1 M KOH aqueous solution by applying a potential step of 1.2 V for 5 min, following a procedure previously described by Anjo et al. [25]. Finally, the electrode was transferred to a supporting electrolyte solution and cycled 10 times between -0.100 V and 1.4 V vs. SCE at a scan rate of $\nu = 0.050 \text{ V s}^{-1}$. The counter electrode was a platinum foil of large area (approx. 2 cm^2). The reference electrode was an aqueous saturated calomel electrode (SCE). Cyclic voltammograms were obtained in a range of scan rates from 0.025 to 0.500 V s^{-1} .

The FIS surface redox couple was mainly studied by SWV. Studies conducted to find the best accumulation potential (E_{acc}) and optimal accumulation time (t_{acc}) to carry out the specific adsorption of FIS on the GC electrode surface are discussed below. The square wave amplitude (ΔE_{SW}) was varied from 0.025 to 0.150 V. The staircase step height (ΔE_s) was 0.005 V. The frequency (f) was varied in a range from 8 to 350 Hz.

The solutions were deaerated by bubbling pure nitrogen for at least 10 min prior to the measurements. The temperature was $20.0 \pm 0.2 \text{ }^\circ\text{C}$.

3. Results and discussion

Fig. 2 shows cyclic voltammograms of FIS on a GC electrode in pH 4.00 buffer solutions. FIS shows three oxidation peaks identified as I, II and III, respectively, in the full range of potential studied (dotted line in Fig. 2). However, the peak I is the FIS main oxidation peak. It shows a quasi-reversible behavior, with an anodic peak potential ($E_{p,a}$) at about 0.340 V vs. SCE. The complementary cathodic peak of peak I showed an increase in the current intensity when the direction of potential sweep was reversed before the onset of peak II (solid line in Fig. 2). The overall profile of cyclic voltammogram of peak I is similar to those previously reported for other related compounds [9,11]. It is well known that the charge transfer process for peak I corresponds to the oxidation of the 3', 4'-dihydroxy group in B ring, giving as product the corresponding quinone species [13,15,26]. Peak II has been ascribed to the oxidation of the reaction product of peak I after an intra-molecular rearrangement [13]. However, theoretical calculations did not allow discarding the fact that a part of quinone species also participates with its C3 hydroxyl group in the second oxidation

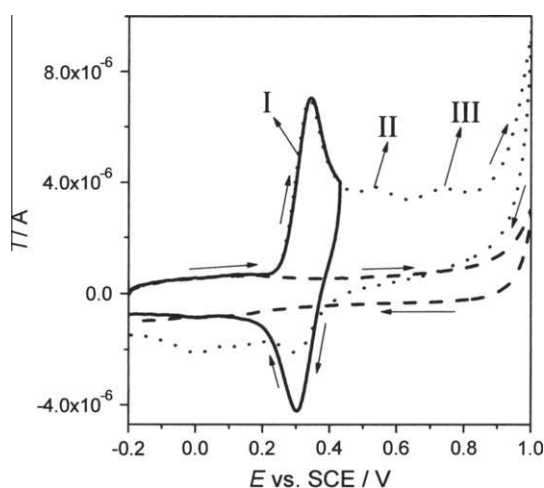


Fig. 2. Cyclic voltammograms of FIS in 15% DMSO + 85% pH 4.00 buffer solution on a GC electrode ($A = 0.070 \text{ cm}^2$) in the full potential range studied (dotted line), and in the potential range corresponding to the first oxidation peak of FIS (solid line). $c_{\text{FIS}} = 1.85 \times 10^{-4} \text{ M}$, $\nu = 0.050 \text{ V s}^{-1}$. The dashed line represents the cyclic voltammogram recorded in the supporting electrolyte solution at the same scan rate. Arrows indicate the direction of potential sweep.

peak before suffering the intra-molecular rearrangement [15]. Peak III corresponds to the oxidation of C7 hydroxy group in A ring (1) [15]. However, Timbola et al. [11] suggested that peaks II and III correspond to the oxidation of the products generated by chemical reactions coupled to the initial charge transfer of peak I.

It is also known that the FIS electrochemical oxidation depends on the pH medium [13,15]. Cyclic voltammograms obtained for the first oxidation peak of FIS at pH 4.00 and 7.00 recorded in solutions in the presence of the antioxidant are shown in Fig. 3a. This oxidation peak was shifted at potentials more positive and the redox couple displayed a greater reversibility as the pH decreased (Fig. 3a). This dependence on pH can be explained considering that the oxidation of phenolic compounds involves proton transfer steps coupled to the charge transfer reactions [27]. On the other hand, the first oxidation peak of FIS showed an adsorption/diffusion mixed control. This behavior could be inferred from a plot of the current function, $\psi = I_{p,a}/A \nu^{1/2}$ vs. $\nu^{1/2}$, where $I_{p,a}$ is the anodic peak current. This plot showed a linear dependence, with an intercept on the y -axis (intercept = $(9 \pm 3) \times 10^{-5} \text{ A cm}^{-2} \text{ V}^{-1/2} \text{ s}^{1/2}$, with $r = 0.9934$), suggesting that both the diffusion and the adsorption of the substrate contribute to the current, i.e. $I_{p,a} = k_1 \nu^{1/2} + k_2 \nu$, where the k 's are some proportionality constants having the appropriate dimensions [28].

The FIS adsorption on GC electrodes was also confirmed when the working electrode was transferred to a supporting electrolyte solution after recording cyclic voltammograms in a solution containing FIS. Therefore, cyclic voltammograms recorded in supporting electrolyte solutions showed a quasi-reversible redox couple well defined at both pH values (Fig. 3b), with current values smaller than those observed in solutions in the presence of FIS. These findings show that both the reactant and the product of the redox couple are adsorbed on the electrode surface, being the surface redox couple slightly better defined at pH 4.00 than pH 7.00.

Other authors previously found a similar behavior when they studied the adsorption of different flavonoids at different pH values. Therefore, Zoulis and Efstathiou [14] studied the pre-concentration of rutin and quercetin on GC electrodes. They found that the optimum pH value was 5.0. Volikakis and Efstathiou [12] also studied the effect of pH on the analytical signal of several flavonoids, including FIS. They also found a greater analytical signal in the acid region, with a maximum at pH 5.0. Markovic et al. [15] also investigated the FIS redox properties at pH 4.0.

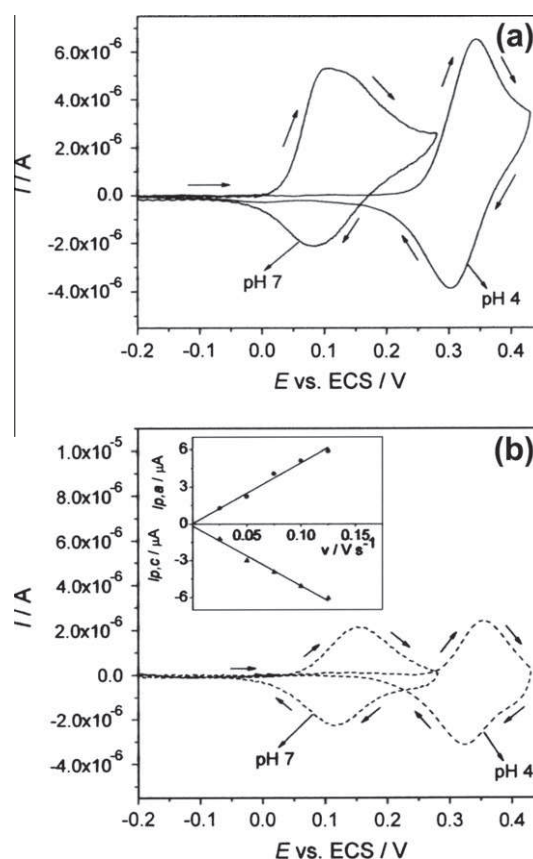


Fig. 3. Cyclic voltammograms of FIS in 15% DMSO + 85% buffer solution at pH values indicated in the figure. (a) Cyclic voltammograms obtained in the presence of FIS (CV₁); (b) cyclic voltammograms (CV₂) obtained in the supporting electrolyte solution after recording CV₁; $c_{\text{FIS}} = 1.85 \times 10^{-4} \text{ M}$, $\nu = 0.050 \text{ V s}^{-1}$. Arrows indicate the direction of potential sweep. Dependence of the anodic peak ($I_{p,a}$) and cathodic peak ($I_{p,c}$) currents of CV₂ voltammograms with the scan rate in 15% DMSO + 85% pH 4.00 buffer solution (inset b).

3.1. Studies related to the FIS adsorption

3.1.1. Cyclic voltammetry

The studies related to the FIS adsorption were performed at pH 4.00. FIS cyclic voltammograms were recorded in solutions containing the antioxidant at a given scan rate, i.e. CV₁. Then, the working electrode was rinsed with the corresponding buffer solution and transferred to another cell containing the supporting electrolyte solution, where a new cyclic voltammogram was obtained at the same scan rate, i.e. CV₂. These cyclic voltammograms (recorded in the supporting electrolyte solution) showed a good reproducibility. Therefore, $I_{p,a}$ values obtained from cyclic voltammograms recorded on three different days under the same experimental conditions, i.e. $c_{\text{FIS}} = 1.85 \times 10^{-4} \text{ M}$ and $\nu = 0.050 \text{ V s}^{-1}$ showed a percent relative standard deviation (%RSD) of 4.8%.

Plots of $I_{p,a}$ vs. ν of CV₂ were linear, with $r = 0.9979$. In addition, plots of cathodic peak currents ($I_{p,c}$) vs. ν were also linear, with $r = 0.9978$ (see inset Fig. 3b). On the other hand, the areas under the oxidation and reduction peaks, after subtracting the background currents, represent the charge (Q) associated with the oxidation of adsorbed FIS (Q_{FIS}) and the reduction of the corresponding adsorbed quinone species (Q_{QUI}), i.e. $Q_{\text{FIS}} = nFA\Gamma_{\text{FIS}}$ and $Q_{\text{QUI}} = nFA\Gamma_{\text{QUI}}$, respectively, where n is the number of exchanged electrons per mole of oxidized or reduced substance, F is the Faraday constant, A is the electrode area and Γ_{FIS} and Γ_{QUI} are the surface concentrations of adsorbed FIS and the corresponding quinone species, respectively. Averages values of $\Gamma_{\text{FIS}} = (3.4 \pm$

$0.3) \times 10^{-10} \text{ mol cm}^{-2}$ were calculated for $n=2$, from cyclic voltammograms recorded in a range of scan rates from 0.025 to 0.125 V s^{-1} . On the other hand, average values of $\Gamma_{\text{QUI}} = (4.7 \pm 0.7) \times 10^{-10} \text{ mol cm}^{-2}$ were determined in the same range of scan rates previously indicated. These findings clearly demonstrate that both FIS and its corresponding quinone species are adsorbed onto the electrode surface. Moreover, values of Γ_{FIS} and Γ_{QUI} are in agreement with those values theoretically expected for the formation of a monolayer of adsorbed substance [29].

These findings show a specific interaction of FIS with the GC electrode surface. In principle, this could be explained by considering that several functional groups, such as phenol, carbonyl and lactone, may be present on an activated GC surface [30]. The interaction between these functional groups and groups present in the chemical structure of FIS might be responsible for the specific adsorption of FIS on GC electrodes.

3.1.2. Square wave voltammetry

The FIS adsorption on GC electrodes was mainly studied by SWV. The forward (I_f), reverse (I_r) and net (I_n) currents obtained from SW voltammograms in the supporting electrolyte solution at pH 4.00 after the FIS accumulation on the GC electrode surface are shown in Fig. 4. Studies were first conducted to find the best accumulation potential (E_{acc}) to perform the FIS adsorption on the electrode surface. Therefore, the E_{acc} was varied from -0.3 to 0.1 V in steps of 0.1 V in the accumulation solution. Then, the SW voltammograms recorded in the supporting electrolyte solution showed no significant differences in the current values as the accu-

mulation potential was varied. On the other hand, no significant difference was observed in the current values of SW voltammograms recorded when the FIS accumulation on the electrode surface was performed at open circuit potential. On the bases of these results, the FIS adsorption was carried out at open circuit potential by immersion of the GC electrode in a FIS solution for a given accumulation time (t_{acc}).

Results shown in Fig. 4 also suggest the quasi-reversible nature of the surface redox reaction, considering the characteristics of voltammetric signals obtained [20]. Experiments performed on the voltammetric peak centered at 0.340 V vs. SCE using different t_{acc} showed an increase in the net peak currents ($I_{p,n}$) with the t_{acc} . The effect of the t_{acc} on $I_{p,n}$ was studied at several FIS concentrations by using SWV. The $I_{p,n}$ increased up to a constant value as the t_{acc} increased for each FIS concentration analyzed. Therefore, constant currents were reached for $t_{\text{acc}} \geq 30 \text{ min}$ for all the FIS concentrations studied. On the other hand, these currents also showed dependence on FIS concentration (results not shown). Maximum constant currents were obtained at a $c_{\text{FIS}}^* \geq 3.5 \times 10^{-6} \text{ M}$. Under these experimental conditions, the electrode surface was completely covered. The shape of SW voltammograms did not show changes with the FIS concentration or the accumulation time (i.e. peak splitting or shifts) for $c_{\text{FIS}}^* \leq 5.0 \times 10^{-6} \text{ M}$, in agreement with that expected for the monolayer coverage on the electrode surface [31].

The adsorption isotherm was derived from the dependence between the fractional coverage of the electrode surface (θ) and c_{FIS}^* . The surface coverage was defined as $\theta = I_{p,n}/I_{p,n,\text{max}}$, where $I_{p,n}$ is

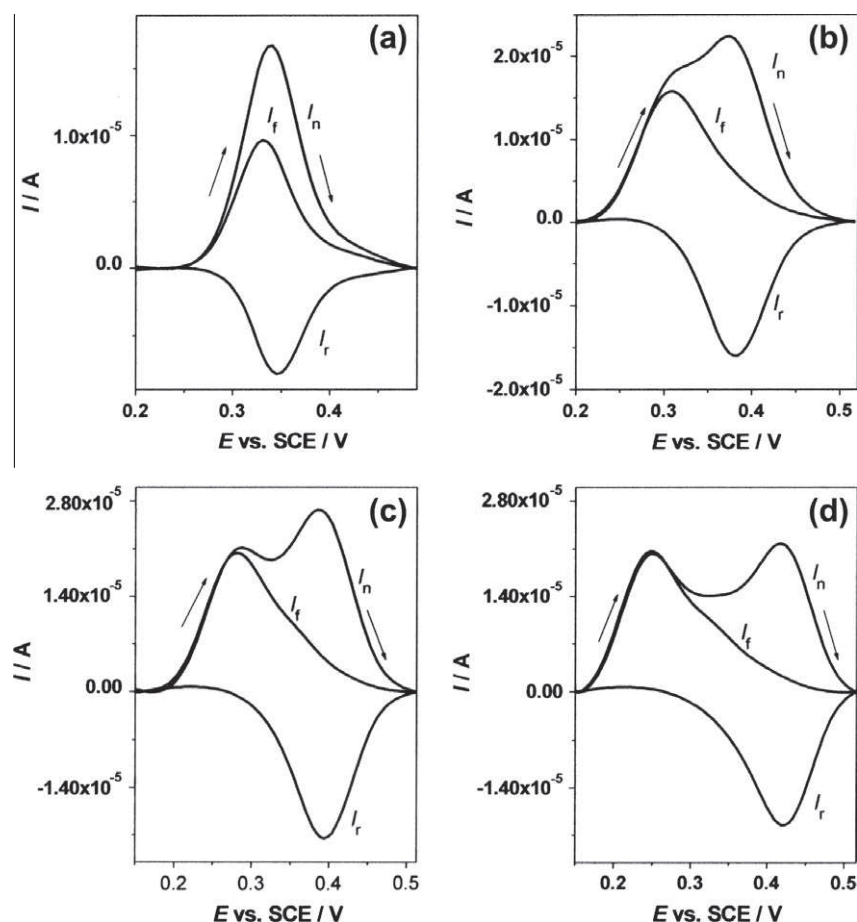


Fig. 4. The forward (I_f), reverse (I_r) and net (I_n) currents from SW voltammograms recorded for FIS adsorbed on a GC electrode recorded in the supporting electrolyte solution (15% DMSO + 85% pH 4.00 buffer solution) at different SW amplitudes. $\Delta E_{\text{SW}} =$ (a) 0.025; (b) 0.075 V; (c) 0.100 and (d) 0.130 V. Other SWV parameters were $\Delta E_s = 0.005 \text{ V}$ and $f = 10 \text{ Hz}$. $c_{\text{FIS}}^* = 1 \times 10^{-6} \text{ M}$. Arrows indicate the direction of potential sweep.

the net peak current from SW voltammograms obtained at a $t_{acc} = 30$ min for different c_{FIS}^* , and $I_{p,n,max}$ is the maximum value of $I_{p,n}$ obtained at the same t_{acc} for the greatest c_{FIS}^* studied, i.e. $c_{FIS}^* = 5.0 \times 10^{-6}$ M.

Experimental results were then fitted in order to find the adsorption isotherm that best describes the specific interaction of FIS with GC electrodes. Therefore, different models of adsorption isotherms were used to perform the fitting, i.e. Langmuir, Temkin, Frumkin, Freundlich. The best fitting of experimental results was achieved when the Frumkin adsorption isotherm was chosen to perform the fit [29,32]. The Frumkin adsorption isotherm can be expressed as [29]:

$$\beta c_{FIS}^* = \frac{\theta}{1-\theta} \exp(g'\theta) \quad (1)$$

where $g' = 2g\Gamma_{FIS,s}/RT$ is the parameter characterizing the interaction among adsorbed species, the g parameter expresses the way in which the adsorption energy changes as a function of the coverage surface. If g is positive, the interactions between adsorbed species are attractive; and if g is negative, the interactions are repulsive. $\Gamma_{FIS,s}$ is the FIS saturation coverage, R and T have their usual meaning, and β is the adsorption coefficient [31,33]. β expresses the strength of adsorption and it is defined as [29]:

$$\beta = \exp\left(\frac{-\Delta G_{ads}}{RT}\right) \quad (2)$$

where ΔG_{ads} is the Gibbs apparent adsorption free energy and the other parameters have the usual meaning [32]. A plot of c_{FIS}^* against θ is shown in Fig. 5a. The fit was performed for values of $0.17 < \theta < 0.79$. The best fit between experimental and theoretical (Eq. (1)) data is shown in Fig. 5a (solid line). For comparison, the fit obtained with the Langmuir isotherm is also shown in Fig. 5a (dotted line).

Values of $g' = (0.31 \pm 0.05)$ and $\beta = 3 \times 10^6$ were obtained from the best fit of experimental data (Eq. (1)). There is a good agreement between experimental data and those obtained from the best fitting, with a $\chi^2 = 1.24 \times 10^{-16}$. The Frumkin adsorption isotherm can provide a useful insight into lateral interactions that may exist within the monolayer. A positive value for the parameter g' indicates that interactions between adsorbed species on the electrode surface are attractive [29,31].

The logarithmic form of the Frumkin adsorption isotherm was also checked. Therefore, a plot of $\ln(\theta/(1-\theta)) + g'\theta$ vs. $\ln c_{FIS}^*$ was linear (Fig. 5b), with a slope of (1.1 ± 0.1) , and an intercept of (16 ± 1) . Values of g' and β similar to those obtained from the fitting were determined, i.e. $g' = 0.29$ and $\beta = 8 \times 10^6$.

The Gibbs apparent adsorption free energy ΔG_{ads} can be calculated from the β value according to Eq. (2). A value of $\Delta G_{ads} = -36$ kJ mol⁻¹ was obtained, which indicates that the overall adsorption process of FIS molecules onto the GC surface is energetically favorable.

3.1.3. Determination of thermodynamic and kinetics parameters of FIS surface redox couple

It is well known that the SWV is a technique suitable for characterizing redox reactions in which the adsorption of reactant and product take place on the electrode surface [20,21].

The theoretical treatment of SWV of redox reactions between reactants and products, which are both adsorbed on electrode surfaces, has been previously discussed [20,21]. Voltammetric responses of these kinds of reactions are characterized by the so called “quasi-reversible maximum” [20,21]. The apparent reversibility of confined redox reactions on the electrode surface depends on the kinetics parameter, κ , defined as the ratio between the formal rate constant (k_s) and the frequency ($\kappa = k_s/f$). The net peak currents from these SW voltammograms are linearly proportional

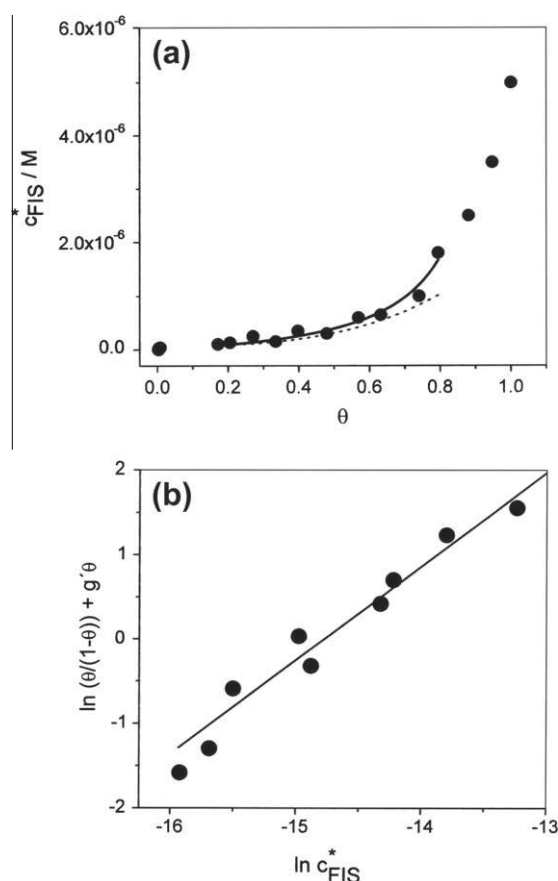


Fig. 5. (a) c_{FIS}^* vs. the surface coverage. (●) Experimental points, (solid line) the best fit obtained from Frumkin isotherm, and (dotted line) the fit line obtained from Langmuir isotherm. (b) Logarithmic form of the Frumkin adsorption isotherm, $r = 0.9765$.

to the frequency, but the factor of this proportionality is a function of the reaction reversibility. If the adsorption of both the reactant and the product of a quasi-reversible surface redox couple is equally strong, a plot of $I_{p,n}f^{-1}$ vs. f or f^{-1} shows a maximum, which appears at a SW frequency that is approximately equal to k_s of the surface redox reaction [20].

The theoretical treatment has also demonstrated that in the region of the maximum of the plot of $I_{p,n}f^{-1}$ as a function of the frequency or the reciprocal of frequency, the curve can be approximated by a parabola [20] and, when $(I_{p,n}f^{-1}) = (I_{p,n}f^{-1})_{max}$, then $f_{max} = k_s/\kappa_{max}$ [20]. Therefore, the equation:

$$k_s = \kappa_{max} f_{max} \quad (3)$$

allows calculating k_s .

The theoretically calculated critical kinetics parameter, κ_{max} , depends on the transfer coefficient, $(1 - \alpha)$, and on the product of the SW amplitude and the number of electrons, $n\Delta E_{SW}$, but it is independent of the normalized potential increment, $n\Delta E_s$, and the amount of substrate initially adsorbed, Γ^* , if there are no interactions among the adsorbed molecules [20]. In the presence of uniform lateral interactions among adsorbed species and following the nomenclature given by Lovric et al. an apparent formal rate constant ($k_{s,app}$) for the surface redox couple is defined as [20,21]:

$$k_{s,app} = k_s \exp(-2a\theta) \quad (4)$$

where $a\theta$ is the interaction product. Thus, the degree of interaction depends on both the fractional coverage of the electrode surface θ

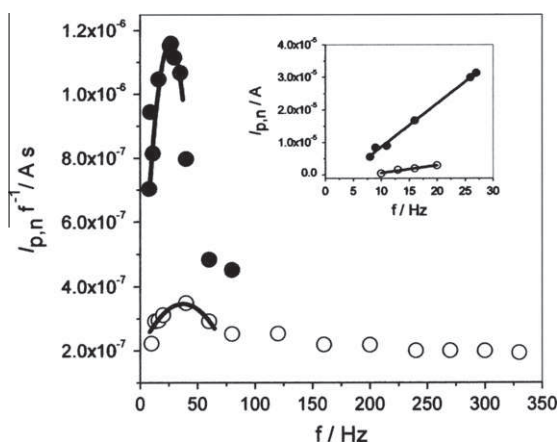


Fig. 6. Dependence of the ratio between net peak currents and the frequency. c_{FIS} : (○): 1×10^{-7} M; (●) 1×10^{-6} M. $\Delta E_{\text{SW}} = 0.025$ V; $\Delta E_s = 0.005$ V. $t_{\text{acc}} = 30$ min. Dependence of net peak currents with the frequency at low frequencies (inset), $r = 0.9828$ (○), and $r = 0.9975$ (●).

and the Frumkin interaction parameter a [20,21]. Therefore, the parameter in Eq. (4) is equivalent to the g' parameter in Eq. (1). Then, to reach the quasi-reversible maximum, the following equation must be satisfied:

$$k_{s,\text{app}}/f_{\text{max}} = (\kappa_{\text{int}})_{\text{max}} \quad (5)$$

where $(\kappa_{\text{int}})_{\text{max}}$ values are identical with κ_{max} values for a simple redox couple without interactions between adsorbed species. In addition, it is possible to infer that when the interaction forces are attractive ($a > 0$) the formal rate constant of the surface redox process decreases.

The net, forward, and reverse currents obtained from typical SW voltammograms of FIS recorded in supporting electrolyte solutions, after the accumulation of FIS on GC electrode surface at open circuit, are shown in Fig. 4.

Plots of $I_{p,n} f^{-1}$ vs. f obtained for two different c_{FIS} are shown in Fig. 6. The critical frequency, f_{max} , decreased as the c_{FIS} increased (Table 1), as it is theoretically expected for a surface redox couple with attractive interactions between adsorbed molecules [20,21]. Plots of $I_{p,n}$ vs. f at low frequencies ($f \leq 30$ Hz) are shown in inset of Fig. 6. A linear dependence was found as it is theoretically expected for a surface redox couple under conditions close to the reversibility [20].

In addition, the theoretical analysis of SW voltammograms of species adsorbed on the electrode surface predicts that for $\log \kappa$ between 0 and 2, the net peak current splits into two and the peak height approximates to zero as the rate of reaction becomes rapid [20,34]. The splitting of the net peak is caused by the skew of forward and reverse peaks on the potential scale as the dimensionless rate constant increases. Experimentally, this behavior may be observed by decreasing the square-wave frequency or increasing the SW amplitude [20,34]. However, changes in the peak shape which carries kinetics information are more effectively produced by varying the SW amplitude at a given frequency [20,34].

Representative SW voltammograms showing net, forward and reverse currents recorded at $f = 10$ Hz and at different ΔE_{SW} are shown in Fig. 4a–d. Thus, for small ΔE_{SW} (Fig. 4a), a single net peak is observed. However, the peak starts to split at about $\Delta E_{\text{SW}} = 0.075$ V (Fig. 4b) and is almost completely split at $\Delta E_{\text{SW}} = 0.130$ V (Fig. 4d). The splitting of net peak is due to the relationship between the rate constant of a surface redox couple and the potential. Therefore, the behavior of the surface redox couple varies from a quasi-reversible to a reversible mechanism as the amplitude of SW increases. An average value of $E_f^0 = (0.340 \pm$

Table 1

Dependence of the maximum frequency with the concentration of FIS in the accumulation solution. $\Delta E_{\text{SW}} = 0.025$ V; $\Delta E_s = 0.005$ V; $t_{\text{acc}} = 30$ min.

$10^6 c_{\text{FIS}}$ (M)	θ	f_{max} (s^{-1})	$k_{s,\text{app}}$ (s^{-1})
0.10	0.26	38	34
0.50	0.50	34	30
0.75	0.64	31	28
1.00	0.87	26	23

Table 2

Anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) peak potentials, the ratio between $I_{p,a}$ and $I_{p,c}$ ($1 - \alpha$) and E_f^0 values for the overall two-electron adsorbed redox couple of FIS at GC electrodes in 15% DMSO + 85% pH 4.00 buffer solution. $\Delta E_s = 0.005$ V. The concentration of FIS in the accumulation solution was $c_{\text{FIS}} = 1 \times 10^{-6}$ M.

f (Hz)	ΔE_{SW} (V)	$E_{p,a}$ (V)	$E_{p,c}$ (V)	E_f^0 (V)	$I_{p,a}/I_{p,c}$	$(1 - \alpha)$
10	0.025	0.331	0.345	0.338	1.23	0.44
	0.075	0.306	0.379	0.342	0.97	0.51
	0.100	0.282	0.394	0.338	0.93	0.52
	0.130	0.248	0.419	0.333	1.05	0.49
20	0.025	0.340	0.345	0.342	1.34	0.41
	0.075	0.321	0.370	0.345	1.01	0.50
	0.100	0.311	0.370	0.340	0.98	0.51
	0.130	0.287	0.389	0.338	0.97	0.51

$$E_f^0 = (0.340 \pm 0.003) \text{ V}; \bar{I}_{p,a}/\bar{I}_{p,c} = (1.1 \pm 0.1); (\bar{1} - \bar{\alpha}) = (0.49 \pm 0.04).$$

0.003 V) for the formal potential of the adsorbed redox couple was estimated from the overall individual formal potentials E_f^0 calculated from the split net peaks at frequencies of 10 and 20 Hz, considering that the two new peaks are symmetrically located around the formal potential of the surface redox couple [20,34]. Values of anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) peak potentials and E_f^0 for each ΔE_{SW} at two different frequencies, and the corresponding overall \bar{E}_f^0 , are shown in Table 2.

It has been reported that the influence of transfer coefficient $(1 - \alpha)$ on ΔE_p is insignificant [20]. However, the shape of the net SW response for an adsorbed reversible redox couple is influenced by the transfer coefficient. For $(1 - \alpha) > 0.2$, the ratio between the forward (anodic) and reverse (cathodic) currents ($I_{p,a}/I_{p,c}$) can be used to calculate $(1 - \alpha)$ through the following equation [35]:

$$\frac{I_{p,a}}{I_{p,c}} = 5.6414 \exp[-3.4606(1 - \alpha)] \quad (6)$$

Experimental values of $I_{p,a}/I_{p,c}$ and the overall anodic transfer coefficient are shown in Table 2. The corresponding average values for these two parameters are $\bar{I}_{p,a}/\bar{I}_{p,c} = (1.1 \pm 0.1)$ and $(\bar{1} - \bar{\alpha}) = (0.49 \pm 0.04)$ for frequencies of 10 and 20 Hz.

Values of $k_{s,\text{app}}$ were calculated through Eq. (5) using both methods previously mentioned for SW voltammetric analysis. The value κ_{max} was obtained from Table 2.3 of Ref. [20], i.e. 0.88 for $n\Delta E_{\text{SW}} = 0.050$ V and $(1 - \alpha) = 0.49$. The error in the estimation of $k_{s,\text{app}}$ using κ_{max} is close to 10% [20].

A plot of $\ln k_{s,\text{app}}$ vs. θ was linear in a range $0.26 < \theta < 0.87$ (four points were included in the regression, $r = 0.9892$). From the intercept and the slope of this plot, values of (0.31 ± 0.06) and 41 s^{-1} were determined for the Frumkin interaction parameter and the overall rate constant, respectively. The value of a is in excellent agreement with that previously determined from the fitting of the best adsorption isotherm ($g' = 0.31$).

The $(1 - \alpha)$ value determined in this study is close to the value of $(1 - \alpha) = 0.54$ obtained by Golabi et al. [13] for the first FIS oxidation peak from CV experiments performed on FIS ethanolic solutions in phosphate buffer solutions of pH 6.00. On the other hand, the value of k_s calculated in this study is about 2.5 times

higher than the value previously reported in the literature [13]. A probable explanation about this behavior would be the different reaction media, and the different electrochemical techniques employed in both studies.

4. Conclusions

The first electrochemical oxidation peak of fisetin is studied in 15% dimethyl sulfoxide +85% pH 4.00 and 7.00 buffer solutions. This oxidation peak showed an adsorption/diffusion mixed control when the oxidation was studied in solutions in the presence of fisetin. The results obtained when studies were carried out in supporting electrolyte solutions demonstrate that both the reactant and the product of the fisetin first oxidation peak are adsorbed at the glassy carbon electrode surface. This surface redox couple was slightly better defined at pH 4.00 than pH 7.00. Frumkin adsorption isotherm was the best to describe the specific interaction of fisetin with glassy carbon electrodes at pH 4.00. Then, square wave voltammetry was used to perform a full characterization of the fisetin overall surface redox couple through the combination of the “quasi-reversible maximum” and the “split of the net square wave voltammetric peak” methods. Therefore, values of E_f^0 , $(1 - \alpha)$, and k_s were determined.

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