



The redox thermodynamics and kinetics of flavonoid rutin adsorbed at glassy carbon electrodes by stripping square wave voltammetry

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ABSTRACT

The adsorptive accumulation of rutin (RU) at glassy carbon (GC) electrodes in 10% ethanol + 90% 1 mol dm⁻³ HClO₄ aqueous solution is studied by using cyclic (CV) and square wave (SWV) voltammetries. The Frumkin adsorption isotherm best described the specific interaction of rutin with carbon electrodes. By fitting the experimental data, values of $-31.9 \text{ kJ mol}^{-1}$ and 0.54 ± 0.02 were obtained for the Gibbs free energy of adsorption and the interaction parameter, respectively. SWV fully characterized the thermodynamics and kinetics of the surface redox process, using a combination of the “quasi-reversible maximum” and the “splitting of SW peaks” methods. Average values of $0.644 \pm 0.003 \text{ V}$ and 0.44 ± 0.02 were obtained for the formal potential and the anodic transfer coefficient, respectively. Moreover, a formal rate constant of $6.1 \times 10^2 \text{ s}^{-1}$ was obtained. SWV was also employed to generate calibration curves. The lowest concentration of RU experimentally measured for a signal-to-noise ratio of 3:1 was $2 \times 10^{-8} \text{ mol dm}^{-3}$ (12 ppb).

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1. Introduction

Polyphenols are one of the most important groups of compounds occurring in plants, in which they are widely distributed. Flavonoids and phenolic acids constitute the most important classes of polyphenols, with more than 5000 compounds already described [1].

Flavonoids exhibit a wide range of biological effects, including anti-bacterial, anti-inflammatory, anti-allergic and anti-thrombotic activities [2]. Flavonoids behave as antioxidants in a variety of ways, including direct trapping of reactive oxygen species, inhibition of enzymes responsible for producing superoxide anions, chelation of transition metals involved in processes forming radicals and prevention of the peroxidation process by reducing alkoxy and peroxy radicals [3].

Rutin (3',4',5,7-tetrahydroxyflavone-3-O-β-D-rutinoside, RU) (Fig. 1) is one of the most bioactive flavonoids [4]. Some investigations showed that RU has a broad range of physiological activities such as anti-inflammatory, anti-tumor and anti-bacterial. Furthermore, it has several pharmacological activities including anti-allergic, vasoactive, anti-viral and anti-protozoal properties.

As an outcome of these biological effects, it has been widely used in treating these diseases. Moreover, it has also been reported that RU has other therapeutic effects such as hypolipidemic, cytoprotective, antispasmodic and anticarcinogenic [4].

Flavonoids have been determined in vegetables and fruit beverages by reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet detection [5,6], coulometric array detection [7] or amperometric electrochemical detection [8]. The possibility of determination of several flavonoids at carbon paste electrodes, such as RU and others, by using adsorptive stripping voltammetry (AdSV) in unstirred solutions [9] and in a flow injection system [10] has been examined. However, fundamental studies on the electrochemical behavior of RU are necessary to assess the feasibility of employing these electroanalytical methods. Some papers have been published about the electrochemical behavior of RU [9,11–17].

The electrochemical oxidation of RU in 0.1 mol dm⁻³ acetate buffer solutions (pH 4.46) at glassy carbon (GC) electrodes has been reported [12]. An adsorptive process was observed from cyclic (CV) and differential pulse (DPV) voltammetries [12]. The anodic oxidation of RU in aqueous buffer solutions of different chemical composition in the pH range from 1.3 to 12.5 by using CV, DPV and square wave voltammetry (SWV) was also reported [13]. The electrochemical oxidation is a complex process, which proceeds in a cascade mechanism, related to the 4'-hydroxyl group present in RU chemical structure (Fig. 1). It has been proposed that the catechol 3',4'-dihydroxyl group is the first to be oxidized by a

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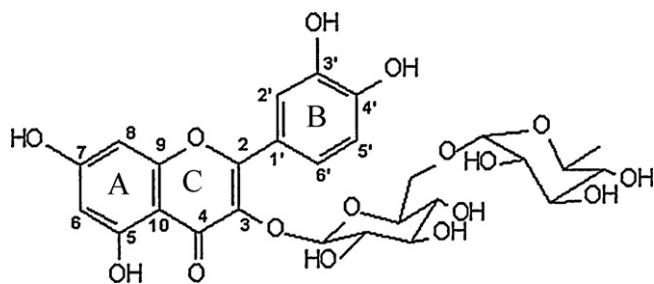


Fig. 1. Chemical structure of rutin.

two-electron–two proton reversible oxidation reaction, followed by an irreversible oxidation reaction due to the 5,7-dihydroxyl group (Fig. 1). Both mechanisms showed to be dependent on pH and an adsorption process was also observed [12,13].

On the other hand, organic compounds which are both surface active and electroactive may be determined at the nanomolar concentration level using stripping voltammetry combined with adsorptive accumulation [18,19]. SWV is a fast and sensitive technique to quantify the amount of initially adsorbed substrate considering its ability to discriminate against capacitive currents [20]. Moreover, SW voltammograms of surface redox processes are highly sensitive to the kinetics of the charge transfer [21–23]. These voltammograms usually show both the so-called “quasi-reversible maximum”, where the ratio between the net peak current ($I_{p,n}$) and the frequency is approximately a parabolic function of the kinetic parameter ω , and the “splitting of the net voltammetric peak” as the SW amplitude increases at a given frequency [21–28]. The theory was initially developed assuming that no interactions exist between immobilized species [23]. However, the case of a kinetically controlled surface redox process accompanied by interactions between adsorbed species has also been studied [23,29]. We have reported the successful application of this methodology to perform a full thermodynamic and kinetics characterization of the surface redox couples of several mycotoxins i.e. altertoxin I, cercosporin and ochratoxin A adsorbed at carbon paste and GC electrodes [30–32]. Recently, we have also used the combination of the “quasi-reversible maximum” and the “splitting of the net voltammetric peak” methods to study the surface quasi-reversible redox couple of morin flavonoid in buffer solutions of pH 7.00 [33].

In this article, we report the results of our studies on the thermodynamics and kinetics of the adsorption of RU at glassy carbon (GC) electrodes in 10% ethanol (EtOH) + 90% 1 mol dm⁻³ HClO₄ aqueous solution. A surface-based quasi-reversible redox couple was obtained from the adsorption of RU, which has been ascribed to an RU type quinone/hydroquinone derivative [13]. This was characterized by CV and SWV techniques. Surface charge transfer kinetics and thermodynamic data for the redox couple were computed by identifying the quasi-reversible maximum and splitting the square wave voltammetric peaks. The adsorption isotherm model that best describes the experimental results is also discussed. These results have very promising analytical applications for determination of RU at trace levels.

2. Experimental

2.1. Reagents

RU obtained from Fluka was used as received. EtOH and water were Sintorgan (HPLC grade). HClO₄ (Merck p.a.) was used as received.

Stock solutions of RU were prepared in EtOH and stored at 5 °C in the dark. They were stable for at least one month. Working solutions were prepared daily by adding aliquots of stock solutions to

1 mol dm⁻³ HClO₄ aqueous solution. In all experiments, the EtOH percentage was constant (10%). The rutin bulk concentration (c_{RU}^*) was varied from 5.0×10^{-8} to 6.1×10^{-5} mol dm⁻³.

2.2. Apparatus and experimental measurements

CV and SWV experiments were carried out with an AutoLab PGSTAT 12 potentiostat, controlled by GPES 4.9 electrochemical software from Eco-Chemie, Utrecht, The Netherlands. In CV measurements, the scan rate (ν) was varied from 0.025 to 1.0 V s⁻¹. For SWV, a square wave amplitude of $\Delta E_{SW} = 0.025$ V and a staircase of $\Delta E_S = 0.005$ V were predominantly employed. The frequency (f) was varied between 10 and 1000 Hz. In some experiments, ΔE_{SW} was varied from 0.025 to 0.125 V.

Electrochemical measurements were performed in a two-compartment Pyrex cell [34]. The working electrode was a GC disk (BAS, 3 mm diameter). It was polished with 0.3 and then 0.05 wet alumina powder (from Fischer), copiously rinsed with water and sonicated in a water bath for 2 min. Then, it was electrochemically activated in aqueous 1 mol dm⁻³ KOH (Merck p.a.) by applying a potential step from 0 to 1.2 V over 5 min, according to a methodology described in literature [35]. Anodic and cathodic peak currents ($I_{p,a}$ and $I_{p,c}$), anodic and cathodic peak potentials ($E_{p,a}$ and $E_{p,c}$) and the difference between anodic and cathodic peak potentials (ΔE_p) were highly reproducible when this pre-treatment was applied to the working electrode. The counter electrode was a large-area platinum foil (approximately 2 cm²). The reference electrode was an aqueous saturated calomel electrode (SCE) fitted with a fine glass Luggin capillary containing the same sample, which is being measured. The temperature was 25.0 ± 0.1 °C. Experimental data were fitted using a nonlinear least squares procedure in Origin 7.0 to determine the adsorption isotherm which best described the specific interaction of RU with GC electrodes. The Chi-square function (χ^2) was used to choose the best fit between experimental and theoretical data.

3. Results and discussion

3.1. Cyclic voltammetry

Cyclic voltammograms of RU at different ν are shown in Fig. 2. Experiments performed on the quasi-reversible voltammetric peak centered at about 0.62 V using different accumulation times (t_{acc})

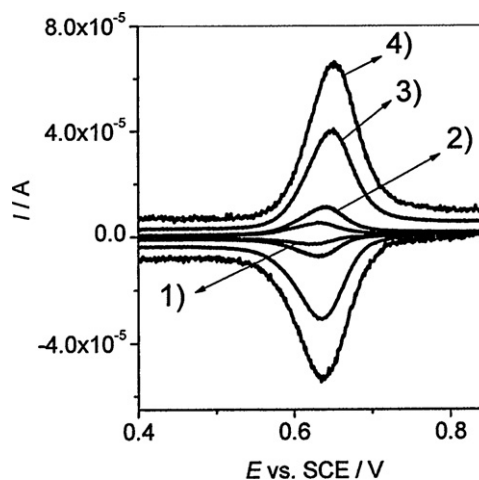


Fig. 2. Cyclic voltammograms of rutin at a GC electrode in 10% EtOH + 1 mol dm⁻³ HClO₄ aqueous solution at different scan rates. $c_{RU}^* = 5.0 \times 10^{-5}$ mol dm⁻³. Reference electrode: SCE. $t_{acc} = 90$ s; $E_{acc} = 0$ V. $\nu =$ (1) 0.050; (2) 0.100; (3) 0.400 and (4) 0.600 V s⁻¹.

Table 1

Peak potentials, peak currents, width at half-height of anodic and cathodic peaks and the difference between anodic and cathodic peak potentials of adsorbed rutin at glassy carbon electrodes in 10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution at different rutin bulk concentrations. $\nu = 0.050$ V s⁻¹. Reference electrode: SCE.

$10^5 \times c_{\text{RU}}^*$ (mol dm ⁻³)	$E_{\text{p,a}}$ (V)	$10^6 \times I_{\text{p,a}}$ (A)	$\Delta E_{\text{p}/2,\text{a}}$ (V)	$E_{\text{p,c}}^{\text{a}}$ (V)	$-10^6 \times I_{\text{p,c}}^{\text{b}}$ (A)	$\Delta E_{\text{p}/2,\text{c}}^{\text{c}}$ (V)	ΔE_{p} (V)
0.005	0.655	0.975	0.059	0.649	0.557	0.048	0.006
0.016	0.658	1.318	0.068	0.649	0.584	0.051	0.009
0.058	0.662	1.544	0.072	0.652	0.667	0.057	0.010
0.15	0.661	1.834	0.078	0.657	0.740	0.057	0.004
0.54	0.664	2.174	0.081	0.655	0.967	0.058	0.009
1.10	0.663	2.571	0.081	0.651	1.230	0.060	0.012
1.68	0.663	2.985	0.080	0.650	1.520	0.063	0.013
3.91	0.661	3.885	0.081	0.648	1.950	0.077	0.013
6.14	0.662	4.259	0.087	0.652	2.070	0.091	0.010

^{a,b} and ^c are the cathodic peak potentials, the cathodic peak currents and the width at half-height of cathodic peaks for the surface redox couple, respectively.

showed an increase in both $I_{\text{p,a}}$ and $I_{\text{p,c}}$, which clearly evidence the surface nature for this electrochemical signal. The optimal t_{acc} was 90 s. The best accumulation potential (E_{acc}) was 0 V. Moreover, when the working electrode was transferred to another electrochemical cell, which contained only the supporting electrolyte (10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution), the quasi-reversible peak system was also observed (results not shown). These results clearly suggest both, the adsorption of RU as well as the quasi-reversible nature of the surface redox reaction by considering the characteristic of voltammetric responses.

Plots of $I_{\text{p,a}}$ vs. ν were linear as it is theoretically expected for a surface redox reaction [36]. The relationship between $I_{\text{p,a}}$ and ν can be expressed as [36]:

$$I_{\text{p,a}} = \left(\frac{n^2 F^2}{4RT} \right) \nu A \Gamma_{\text{RU}} \quad (1)$$

where n is the number of electron exchanged per mole of oxidized substance, F is the Faraday constant, A is the microscopic electrode area and Γ_{RU} is the surface concentration of adsorbed RU. From the slope of $I_{\text{p,a}}$ vs. ν plots obtained at a $c_{\text{RU}}^* = 5.0 \times 10^{-5}$ mol dm⁻³ (slope $(104 \pm 1) \times 10^{-6}$ A s V⁻¹, linear correlation coefficient, $r = 0.9995$), a value of $\Gamma_{\text{RU}} = 7.6 \times 10^{-10}$ mol cm⁻² was obtained. This value is in a good agreement with the maximum surface coverage determined by Kang et al. [12] through the area of oxidation peaks, where the associated charge (Q) can be expressed according to the Faraday law as $Q = nFA\Gamma_{\text{RU}}$. On the base of the value obtained for the maximum surface coverage these authors proposed that RU uses the benzene ring with 3',4'-dihydroxides adsorbed on the GC electrode surface, while the other parts of the molecule keep away from the electrode surface.

The average experimental ΔE_{p} value was (0.012 ± 0.003) V and the average width at half-height of the anodic peak ($\Delta E_{\text{p}/2,\text{a}}$) was (0.069 ± 0.004) V in all range of scan rates studied when c_{RU}^* was 5.0×10^{-5} mol dm⁻³. ΔE_{p} and $\Delta E_{\text{p}/2,\text{a}}$ were practically independent of the scan rates for all concentrations studied. Moreover, ΔE_{p} and $\Delta E_{\text{p}/2,\text{a}}$ also showed an almost negligible dependence on c_{RU}^* , where an average value of $\Delta E_{\text{p}} = (0.009 \pm 0.005)$ V was determined at $\nu = 0.050$ V s⁻¹ for all concentrations studied (Table 1).

However, $\Delta E_{\text{p}/2,\text{a}}$ slightly increased as the c_{RU}^* was increased. It is greater than that theoretically expected for a 2e⁻ redox reaction. When there are no lateral interactions between surface-confined redox centers and a rapid equilibrium is established with the electrode surface, a zero peak-to-peak splitting ($\Delta E_{\text{p}} = 0$) and a $\Delta E_{\text{p}/2,\text{a}} = 0.0453$ V at 25 °C are expected for a two-electron transfer [36]. Our observation that ΔE_{p} was simultaneously nonzero and independent of the scan rates, suggests that slow charge transfer kinetics are not the origin of the responses found. Slow kinetics would cause an increase in ΔE_{p} with higher scan rates. Ohmic effects are neither an explanation for the finite scan rate indepen-

dent ΔE_{p} because a higher ohmic drop would be expected at higher scan rates due to the passing of a higher total current. Moreover, diffusion is effectively excluded as an explanation considering that the peak current increased linearly with ν at all concentrations and scan rates studied, rather than with $\nu^{1/2}$ as expected for diffusion-controlled reactions. There have been previous reports of finite ΔE_{p} values that are independent of the scan rate for surface-confined redox couples [37]. This unusual quasi-reversibility or apparent non-kinetic hysteresis in cyclic voltammetry has been interpreted in terms of N-shaped free energy curves. In this interpretation, unusual hysteretic quasi-reversibility is a non-equilibrium behavior, in which a finite ΔE_{p} is observed because some rate processes are slow on the time scale of the experiment [38].

3.2. Square wave voltammetry

3.2.1. Adsorption isotherm

The forward (I_{f}), reverse (I_{r}) and net (I_{n}) currents obtained from SW voltammograms of RU in 10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution at a GC electrode are shown in Fig. 3. These results clearly suggest both, the adsorption of RU and the quasi-reversible nature of the surface redox couple by considering the characteristic of I_{f} , I_{r} and I_{n} voltammetric responses obtained. The selective interaction of RU with the GC electrode surface could be explained by considering that several oxygen containing functional groups, such as carboxylic acid, lactone, *o*-quinone, *p*-quinone, carbonyl, phenol, etc., may be present on an activated GC electrode [39]. The interaction between these groups and the -OH groups present in the chemical structure of RU might be responsible for the adsorption of RU on GC electrodes. Similar results were obtained in our laboratory for the surface behavior of altetroxin I on carbon paste electrodes [30] and cercosporin, ochratoxin A and morin on GC electrodes [31–33].

A plot of the net peak currents ($I_{\text{p,n}}$) obtained from SW voltammograms at different c_{RU}^* is shown in Fig. 4a. Steady state currents were obtained for $c_{\text{RU}}^* \geq 4 \times 10^{-5}$ mol dm⁻³. The adsorption isotherm was derived from the dependence between the fractional coverage of the electrode surface (θ) and c_{RU}^* . The surface coverage was defined as $\theta = I_{\text{p,n}}/I_{\text{p,n,max}}$ where $I_{\text{p,n}}$ are the net peak currents from SW voltammograms obtained at $t_{\text{acc}} = 90$ s and $E_{\text{acc}} = 0$ V for different c_{RU}^* and $I_{\text{p,n,max}}$ and is the maximum value of $I_{\text{p,n}}$ obtained at the same t_{acc} and E_{acc} for the greatest c_{RU}^* studied. A plot of c_{RU}^* vs. θ is shown in Fig. 4b. Fits of different adsorption isotherms (Langmuir, Frumkin, Temkin and Freundlich) were tested to describe the adsorption of RU at GC electrodes. The best fit was obtained with the Frumkin adsorption isotherm, which can be expressed as [36]:

$$\beta c_{\text{RU}}^* = \frac{\theta}{1-\theta} \exp(g'\theta) \quad (2)$$

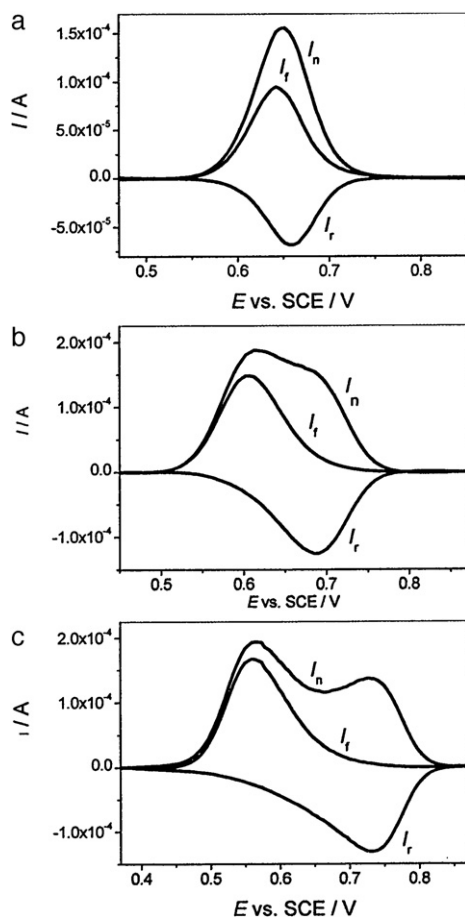


Fig. 3. The forward (I_f), reverse (I_r) and net (I_n) currents from SW voltammograms of rutin adsorbed at glassy carbon electrodes in 10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution at different SW amplitudes. $\Delta E_{SW} =$ (a) 0.025; (b) 0.075 and (c) 0.125 V. $c_{RU}^* = 2.5 \times 10^{-5}$ mol dm⁻³. $\Delta E_s = 0.005$ V and $f = 100$ Hz.

where $\beta = \exp(-\Delta G_{ads})$ is the adsorption coefficient, which expresses the strength of adsorption, $g' = 2g\Gamma_{RU}/RT$ is a parameter characterizing the interaction between the adsorbed molecules and ΔG_{ads} is the Gibbs free energy of adsorption. The fit was performed for values of $0.1 < \theta < 0.8$ (line solid in Fig. 4b). From the best fit, the parameters were: $\beta = (3.8 \pm 0.2) \times 10^5$, $g' = (0.54 \pm 0.02)$ and $\chi^2 = 1.7 \times 10^{-14}$. There is an excellent agreement between experimental data and results of the fit. A value of $\Delta G_{ads} = -31.9$ kJ mol⁻¹ was calculated for the standard free energy of adsorption, which indicates that the overall adsorption process of RU onto the GC surface is energetically favorable. In addition, a positive value for g' indicates that interactions between adsorbed molecules on the electrode surface are attractive. This behavior may be explained by considering the possibility to form inter-molecular hydrogen bonds between -OH groups present in RU chemical structure (Fig. 1).

3.2.2. Determination of thermodynamic and kinetics parameters of rutin adsorbed at glassy carbon electrodes

Plots of $I_{p,n}f^{-1}$ vs. f for RU adsorbed at GC electrode surfaces at different t_{acc} (at a given c_{RU}^*) as well as at different c_{RU}^* are shown in Fig. 5. The apparent reversibility of a confined redox couple at the electrode surface depends on the kinetic parameter (ω) defined as the ratio between the surface standard rate constant (k_s) and the frequency ($\omega = k_s f^{-1}$). Net peak currents from these SW voltammograms are linearly proportional to the frequency, but the factor of this proportionality is a function of the reversibility of the reac-

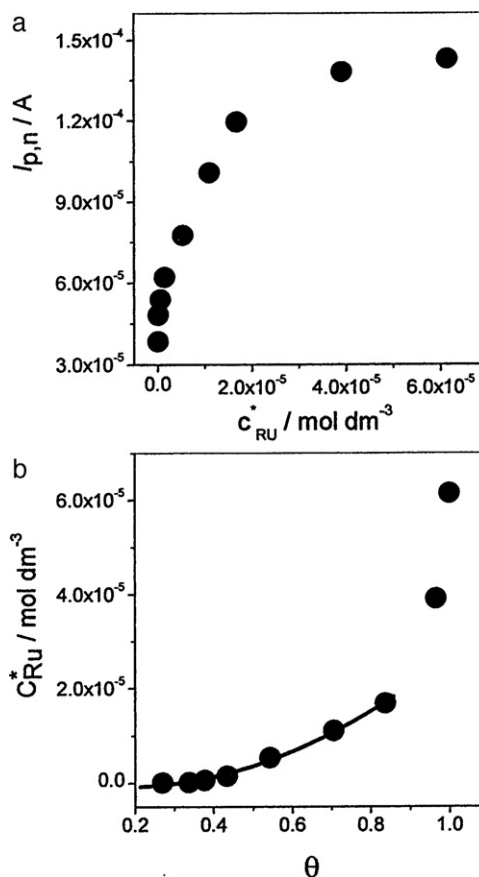


Fig. 4. (a) Plot of the net peak current ($I_{p,n}$) vs. the rutin bulk concentration (c_{RU}^*) and (b) Experimental data compared with the best-fit by using Frumkin adsorption isotherm model. (●) experimental points, (-) results of the best fit. $\Delta E_{SW} = 0.025$ V; $\Delta E_s = 0.005$ V and $f = 100$ Hz.

tion. If the adsorption of both the reactant and the product of a quasi-reversible redox couple are equally strong, a maximum usually appears in the plot of $I_{p,n}f^{-1}$ vs. f or f^{-1} , which appears at a frequency which is approximately equal to k_s of the redox couple [23,26]. In the maximum region, the curve can be approximated by a parabola, and if $(I_{p,n}f^{-1} \text{ vs. } f) = (I_{p,n}f^{-1} \text{ vs. } f)_{max}$, then $f_{max} = k_s/\omega_{max}$ [21,23]. Therefore, k_s can be determined from the following equation:

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

Theory also predicts that a useful “quasi-reversible maximum” appears only if $-1.5 < \log \delta < 1.5$ [23,26], where δ is the ratio between the adsorption constants of the oxidized and reduced forms of the surface redox couple.

Table 2
Dependence of the maximum frequency with the accumulation time and the RU concentration. $\Delta E_{SW} = 0.025$ V; $\Delta E_s = 0.005$ V.

$10^6 \times c_{RU}^*$ (mol dm ⁻³)	t_{acc} (s)	θ	f_{max} (s ⁻¹)	$k_{s,app}$ (s ⁻¹) ^a
0.5	30	0.101	663	587
0.5	60	0.169	593	525
5.0	10	0.480	505	447
5.0	60	0.520	486	430
25	90	0.910	224	198

^a $k_{s,app} = f_{max} \omega_{max}$, where $\omega_{max} = 0.885$ was obtained from Table 2.3 of Ref. [23] for $n\Delta E_{SW} = 0.050$ V and $(1 - \alpha) = 0.44$.

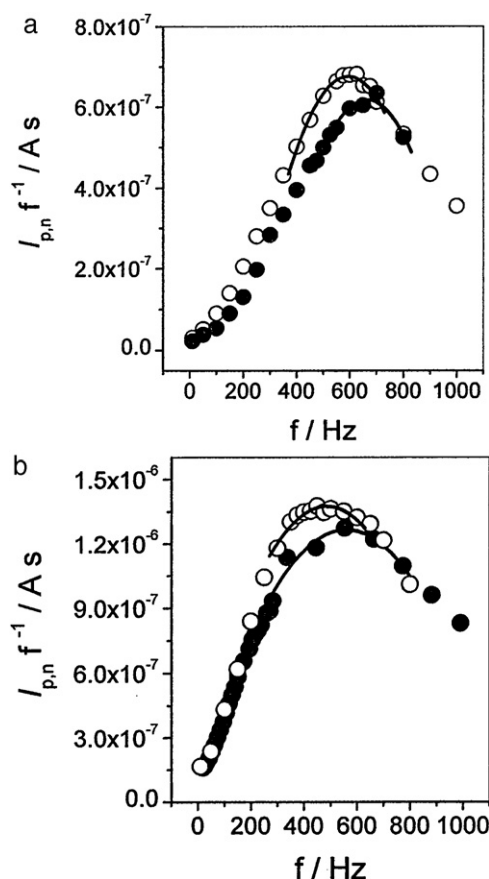


Fig. 5. Dependence of the ratio between the net peak current ($I_{p,n}$) and the frequency with the frequency obtained for rutin adsorbed at glassy carbon electrodes in 10% EtOH + 1 mol dm⁻³ HClO₄ aqueous solution. (a) t_{acc} : (●) = 30 s; (○) = 60 s ($c_{RU}^* = 5 \times 10^{-7}$ mol dm⁻³); (b) t_{acc} : (●) = 10 s; (○) = 60 s ($c_{RU}^* = 5 \times 10^{-6}$ mol dm⁻³). $\Delta E_{SW} = 0.025$ V; $\Delta E_s = 0.005$ V.

The theoretically calculated critical kinetics parameter, ω_{max} , depends on the anodic 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 initially adsorbed reactant [23,26]. The development from now on assumes that surface reaction involves uniform lateral interactions between reactant and product immobilized onto the electrode surface. Thus, in the presence of uniform lateral interactions between adsorbed species, the product of k_s and the exponential term $\exp(-2g'\theta)$ defines a new apparent rate constant of the surface redox reaction [23,29]:

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

where $g'\theta$ is the interaction product. Therefore, the degree of interactions depends on both the relative coverage of the electrode surface θ and Frumkin interaction parameter g' [23,29]. The position of the maximum is associated with a certain critical value of the interaction product $(g'\theta)_{max}$, which depends on the value of the ratio k_s/f . Therefore, to reach the quasi-reversible maximum, the following condition must be satisfied:

$$k_s \exp(-2g'\theta) f_{max}^{-1} = (\omega_{int})_{max} \quad (5)$$

where the values of $(\omega_{int})_{max}$ are identical with ω_{max} for a simple surface redox reaction without interactions between adsorbed species [23]. Moreover, as $k_{s,app} = k_s \exp(-2g'\theta)$, it is possible to infer that when the interaction forces are attractive ($g' > 0$) the formal rate constant for the surface redox couple decreases. As it can be observed in Fig. 5, the f_{max} decreases with both the t_{acc} and the c_{RU}^* as it is theoretically expected for a surface redox couple with attractive interactions between adsorbed molecules [23,29] (see Table 2).

Theory also predicts that if the rate of reaction becomes rapid, the net peak current of a SW voltammograms splits and the peak height approaches to zero [25,27]. 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. This behavior may be observed by decreasing the SW frequency or by increasing the SW magnitude [25,27]. However, changes in the peak shape, which carry kinetics information, are more effectively produced by varying the SW amplitude rather than varying the frequency [25]. Therefore, a set of SWV experiments were performed, where c_{RU}^* and $n\Delta E_{SW}$ were varied at a given frequency. The I_f , I_r and I_n obtained at $f = 100$ Hz for a given c_{RU}^* at different $n\Delta E_{SW}$ are shown in Fig. 3. Therefore, for small ΔE_{SW} (i.e., 0.025 V) and $f = 100$ Hz, a single net peak was found (Fig. 3a). However, the peak started to split at $\Delta E_{SW} = 0.075$ V (Fig. 3b) and was almost completely split at $\Delta E_{SW} = 0.125$ V (Fig. 3c). The peak split is due to the

Table 3

Anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) peak potentials, anodic ($I_{p,a}$) and cathodic ($I_{p,c}$) peak currents for the splitted SW peaks, 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 rutin at GC electrodes in 10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution.

$10^4 \times c_{RU}^a$ (mol dm ⁻³)	f (Hz)	ΔE_{SW} (V)	$E_{p,a}$ (V)	$I_{p,a}$ (μ A)	$E_{p,c}$ (V)	$-I_{p,c}$ (μ A)	$I_{p,a}/I_{p,c}$	$(1 - \alpha)$
0.25	40	0.050	0.617	36.72	0.666	32.29	1.14	0.47
		0.075	0.593	39.70	0.686	34.25	1.16	0.46
		0.100	0.569	41.50	0.710	34.46	1.20	0.45
		0.125	0.544	42.88	0.735	34.14	1.25	0.44
		0.150	0.520	43.73	0.759	33.27	1.32	0.43
		0.175	0.495	44.56	0.788	32.29	1.38	0.41
0.50	40	0.050	0.622	49.99	0.666	42.91	1.16	0.46
		0.075	0.598	53.56	0.686	46.70	1.15	0.47
		0.100	0.573	55.98	0.710	47.43	1.18	0.46
		0.125	0.549	58.11	0.735	47.00	1.24	0.45
		0.150	0.525	59.65	0.759	45.98	1.29	0.43
		0.175	0.500	60.87	0.783	44.04	1.38	0.41
0.50	100	0.050	0.632	170.00	0.671	143.20	1.19	0.46
		0.075	0.612	195.70	0.686	165.60	1.18	0.46
		0.100	0.588	211.90	0.725	169.40	1.24	0.45
		0.125	0.569	220.70	0.725	169.40	1.30	0.43
		0.150	0.544	228.30	0.754	167.40	1.36	0.42
		0.175	0.515	227.00	0.779	165.30	1.37	0.42

^a c_{RU}^* is the rutin bulk concentration. $E_f^0 = (0.644 \pm 0.003)$ V; $(1 - \alpha) = (0.44 \pm 0.02)$.

relationship between the potential-dependent rate constant for the redox couple and the time scale of the experiment. An average value of (0.644 ± 0.003) V for the overall formal potential (\overline{E}_f^0) of the adsorbed two-electron redox couple was estimated from: $(\overline{E}_f^0) = 1/2(E_{p,f} + E_{p,r})$ [19], where $E_{p,f}$ and $E_{p,r}$ are peak potentials for the forward (anodic) and reverse (cathodic) scans, respectively.

Moreover, the shape of the net SW response for an adsorbed quasi-reversible redox couple is influenced by the transfer coefficient [23,26,40]. For $(1 - \alpha) > 0.2$, the ratio between the forward (anodic) and reverse (cathodic) peak currents, $I_{p,a}/I_{p,c}$, can be expressed as [23,40]:

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

Experimental values of $E_{p,a}$, $E_{p,c}$, $I_{p,a}$, $I_{p,c}$, $I_{p,a}/I_{p,c}$ and $(1 - \alpha)$ are shown in Table 3. Average values of $(I_{p,a}/I_{p,c}) = (1.25 \pm 0.08)$ and $(1 - \alpha) = (0.44 \pm 0.02)$ were obtained for frequencies of 40 and 100 Hz.

Values of $k_{s,app}$ were calculated from Eq. (3) using the values of ω_{max} obtained from Table 2.3 of Ref. [23] (see Table 2), i.e., 0.885 for $n\Delta E_{SW} = 0.050$ V and $(1 - \alpha) = 0.44$ (Table 3). The error in the estimation of $k_{s,app}$ by using ω_{max} is close to 10% [23].

A plot of \ln Eq. (4) was linear in the range $0.101 < \theta < 0.520$ (four points were included in the correlation, $r = 0.9812$). From the intercept and the slope of this plot, values of $6.1 \times 10^2 \text{ s}^{-1}$ and (0.33 ± 0.09) were calculated for k_s and g' , respectively. The value of g' is close to that previously determined from the fitting of adsorption isotherm (Section 3.2.1) which indicates that the proposed kinetic approach based on uniform lateral interactions is reasonable to obtain the kinetic parameters.

The k_s value obtained indicates that the kinetics of adsorbed RU is close to that obtained in our laboratory for the mycotoxin altertoxin I ($k_s = 6.8 \pm 0.3 \times 10^2 \text{ s}^{-1}$) in 20% acetonitrile + 1 mol dm⁻³ HClO₄ at carbon paste electrode [30]. However, RU shows a faster kinetics than cercosporin ($k_s = 3.5 \pm 0.5 \times 10^2 \text{ s}^{-1}$) in 1 mol dm⁻³ HClO₄ aqueous solutions at glassy carbon electrodes [31], ochratoxin A ($k_s = 11 \text{ s}^{-1}$) in 10% acetonitrile + 90% 1 mol dm⁻³ HClO₄ [32] and morin ($k_s = 87 \text{ s}^{-1}$) in 0.2 mol dm⁻³ phosphate buffer solutions of pH 7.00 [33] at GC electrodes.

3.2.3. Determination of rutin in pure solutions

Some SWV results for RU adsorbed as a function of c_{RU}^* are shown here as an introduction for a future publication about its analytical applications. The net current–potential curve in SWV is the most useful analytical signal for quasi-reversible redox couples as a consequence of the contribution of both forward and backward currents to net currents as it is the case for the quasi-reversible signal obtained for rutin (Section 3.2.1, Fig. 3). The combination of adsorptive accumulation with SWV provides to be an electroanalytical tool which is very valuable for carrying out trace analysis of compounds that are both surface active and electroactive. The determination of RU was performed at GC electrodes in unstirred 10% EtOH + 90% 1 mol dm⁻³ HClO₄ aqueous solution after a $t_{acc} = 90$ s and $E_{acc} = 0$ V. The linear regression between $I_{p,n}$ and c_{RU}^* at a $f = 100$ Hz can be expressed by a least square procedure as:

$$I_{p,n} = (5.1 \pm 0.2) \times 10^{-5} + (4.6 \pm 0.4)c_{RU}^* \quad r = 0.9907 \quad (7)$$

with a relative standard deviation, RSD = 8.7%. In Eq. (7), $I_{p,n}$ and c_{RU}^* are expressed in amperes and mol dm⁻³, respectively. Data used in the regression analysis of the calibration curve were the average of two or three replicated measurements (eight experimental points were taken into account). The calibration curve was linear in the concentration range from 1.62×10^{-7} to 1.1×10^{-5} mol dm⁻³. Moreover, the lowest concentration value measured experimentally for a signal-to-noise ratio of 3:1 was 2×10^{-8} mol dm⁻³

($12 \mu\text{g L}^{-1}$). The detection limit determined by us from adsorptive SWV is quite similar to those obtained by DPV on GC and carbon paste electrodes [9,10,12] but significantly lower than that informed in literature by using isocratic RP-HPLC [6]. Results obtained in this work and those obtained previously by us on other matrixes [31,41] clearly establish a challenge to develop a new methodology for the determination of RU in real samples based on adsorptive SWV, taking into account that RU is usually found in pharmaceutical formulations and biological fluids, with the presence of possible main interference of other flavonoids, ascorbic acid and uric acid.

4. Conclusions

The combination of the “quasi-reversible maximum” and the “split of net SW peaks” methods were employed for the first time to carry out a full thermodynamic and kinetics characterization of rutin adsorbed at glassy carbon electrodes. The Frumkin adsorption isotherm was the best to describe the specific interaction of rutin with glassy carbon electrodes. On the other hand, adsorptive square wave voltammetry performed on rutin accumulated on glassy carbon electrodes appears as a very promising analytical tool for the determination of rutin in real samples.

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