

CRITICAL ASPECTS IN EXPLORING TIME ANALYSIS FOR THE VOLTAMMETRIC ESTIMATION OF KINETIC PARAMETERS OF SURFACE ELECTRODE MECHANISMS COUPLED WITH CHEMICAL REACTIONS

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The accurate determination of the rate constant related to the electron transfer step of so-called “surface redox active compounds” by voltammetric measurements is very important because it is linked to the reactivity and stability of many biological and chemical systems such as redox enzymes, vitamins, hormones, and many more. Evaluation of the kinetics of the electron transfer is often challenging, especially when chemical equilibria are coupled to the electron transfer step. In this work, we theoretically consider some critical aspects of the time-related methodologies in square-wave voltammetry (SWV), which is designed to analyze the kinetics of the electron transfer step of surface mechanisms coupled with chemical reactions. We demonstrate with a series of simulated scenarios that caution must be taken when exploring the time-related analysis for kinetic characterizations for both surface CE and EC mechanisms. The main concern stems from the fact that the SW frequency simultaneously affects both the kinetics of electron transfer and that of chemical reactions as well. Under defined conditions, the SW frequency variation in the case of surface EC and CE mechanisms may produce unexpected features of the voltammetric patterns. In many scenarios, time-independent analysis, such as those related to the square-wave amplitude and potential increment, are seen as alternative tools to evaluate the rate parameter of electrode reactions.

Keywords: kinetics of electron transfer; surface EC_{rev} mechanism; surface C_{rev}E mechanism; quasi-reversible maximum; square-wave voltammetry

КРИТИЧКИ АСПЕКТИ ВО ПРИМЕНАТА НА ВРЕМЕНСКИ-ЗАВИСНИТЕ АНАЛИЗИ ВО ВОЛТАМЕТРИСКИТЕ МЕТОДИ ЗА ОПРЕДЕЛУВАЊЕ НА КИНЕТИЧКИТЕ ПАРАМЕТРИ КАЈ ПОВРШИСКИ ЕЛЕКТРОДНИ МЕХАНИЗМИ ШТО СЕ ПОВРЗАНИ СО ХЕМИСКИ РЕАКЦИИ

Прецизното определување на константите на брзина на трансфер на електрони кај „површинско-активните системи“ со примена на волтаметриски методи е од големо значење, бидејќи вредностите на овие константи се директно поврзани со реактивноста, и стабилноста на голем број важни биолошки и хемиски системи како што се „редокс“ ензимите, витамините, хормоните и многу други супстанции. Определувањето на кинетиката на електронски трансфер многу често е комплексен процес, посебно кога процесот на електронски трансфер е поврзан со хемиски рамнотежи. Во овој труд, анализирани се повеќе критични аспекти на временско-зависни анализи во услови на квадратно-бранова волтаметрија, (SWV) дизајнирани за анализа на кинетиката на електронски трансфери на површински електродни механизми што се

комплицирани со хемиски реакции. Преку теоретските резултати од голем број симулации, покажано е дека кога се работи за површински електродни механизми комплицирани со претходна или последователна хемиска реакција (CE и EC механизми), потребно е со доза на голема претпазливост да се употребуваат временски-зависните анализи за определување на кинетиката на електронските трансфери. Главниот проблем е што кај ваквите механизми, фреквенцијата во квадратно-брановата волтаметрија истовремено влијае и врз кинетиката на електронскиот трансфер, но и врз кинетиката на хемиските реакции што се поврзани со електронскиот трансфер. При определени услови, фреквенцијата во SWV, кај површински EC и CE механизми, може да предизвика неочекувани својства во волтаметриските одговори. Во голем број на експериментални ситуации, потребно е да се употребат алтернативни и временски-независни анализи за определување на кинетиката на електронски трансфер, методи базирани на својствата на волтаметриските одговори на овие системи како функција од квадратно-брановата амплитуда или потенцијалниот инкремент.

Клучни зборови: кинетика на електронски трансфер; површински EC_{rev} механизам; површински $C_{rev}E$ механизам; квази-реверзибилен максимум; квадратно-бранова волтаметрија

1. INTRODUCTION

Cyclic voltammetry (CV) [1–3] and square-wave voltammetry (SWV) [4–8] are recognized as key electrochemical techniques for getting insights into electrode mechanisms, as well as for accessing kinetic and thermodynamic parameters of many biological and chemical systems. Both techniques are widely utilized in various areas of physics, chemistry, pharmacy, medicine, and biology. From the characteristics of cyclic and square-wave voltammograms, relevant information can be derived on adsorption, mass-transport, and charge-transfer phenomena that take place at the electrode/electrolyte interface [1–8]. Moreover, both techniques are very suitable for analyzing relevant aspects of chemical reactions that are coupled to the electron transfer process [1, 4]. Analyzing these reactions is quite pertinent because they mimic important physiological processes, especially those in enzymatic biochemistry [9–15].

The theory of voltammetry aims to reveal the key parameters related to the kinetics and thermodynamics of electrode mechanisms which control the main features of the outcome of the voltametric experiment. Estimation of the magnitude of the standard rate constant of the electron transfer step k_s^\ominus , most frequently considered in the frame of the Butler-Volmer formalism, encompasses one of the most important applications of CV and SWV [1–8]. The correlation of k_s^\ominus with the energy difference of electrons between the orbitals of the working electrode and those of the analyzed electroactive species is critical for understanding the kinetics of many processes encountered in the redox chemistry of various systems [1, 2].

By combining the Butler-Volmer equation with modified Fick's laws for electrochemical reac-

tions of dissolved species that are coupled with chemical reactions, one obtains implicit expressions that link the magnitude of the Faradaic current (I) and the applied potential (E), including parameters related to the kinetics and thermodynamics of the involved chemical processes. Theoretical simulation protocols under voltammetric conditions frequently involve dimensionless kinetic parameters. Such parameters are actually sort of normalized functions of the real rate constants (e.g., diffusion coefficient (D), electrochemical standard rate constant (k_s^\ominus), chemical rate constant (k) and the critical time parameters of a particular voltametric experiment (e.g., frequency (f) in SWV or scan rate (v) in CV) [1, 2, 4, 5]. A typical example is the electrode kinetic parameter of a quasi-reversible electrode reaction of a dissolved redox couple, defined as $K_{ET} = k_s^\ominus / [(Df)^{1/2}]$, where the standard rate constant k_s^\ominus , the diffusion coefficient D , and the time parameter f are combined into a single parameter which predominantly determines electrochemical reversibility of an electrode reaction. In such an approach, the interpretation of theoretical data is generalized, revealing that the voltametric outcome depends on the specific interrelation between the real system parameters.

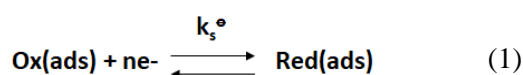
For the majority of electrode processes, access to the kinetic parameters coupled with chemical equilibria is obtained by varying the scan rate (v , in CV) or frequency (f in SWV) [1–9]. However, such an approach hides the implicit danger related to the accuracy of the kinetic parameters obtained. This comes from the fact that the voltametric response depends on several dimensionless parameters that are related to both electrode and chemical kinetics, while the time parameter is incorporated in all dimensionless kinetic parameters. Consequently, by varying time of the voltammetric

experiment (i.e., v or f in CV and SWV, respectively), one simultaneously affects the kinetics of all time-dependent parameters. This, in turn, can lead to misleading interpretations and erroneous estimations of the kinetic data. In this theoretical work, we illustrate this phenomenon by considering a few surface electrode mechanisms coupled with chemical reactions under the conditions of SWV. The aim of the study is to elucidate the best approach for analyzing voltammetric data for the purpose of adequate kinetic analysis when complex electrode mechanisms are considered.

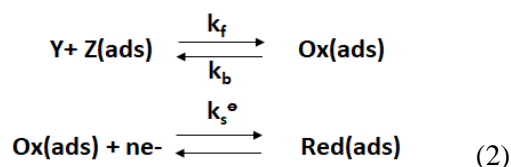
2. THEORETICAL MODELS

Theoretical aspects of three, diffusionless, surface-confined electrode mechanisms are considered. They are described by the following reaction schemes:

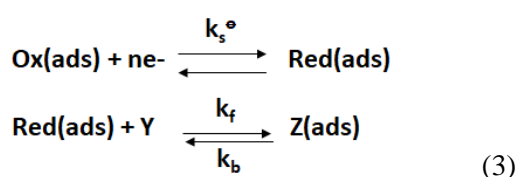
A. Simple surface electrode reaction (E)



B. Surface electrode reaction coupled with a preceding, reversible chemical reaction (surface $C_{\text{rev}}E$ mechanism)



C. Surface electrode reaction associated with a reversible follow-up chemical reaction (surface EC_{rev} mechanism)



It is assumed that all immobilized species are distributed in the form of a monolayer on the working electrode surface. In addition, no lateral interactions of any kind exist between the immobilized species. The relevant recurrent formulas for calculating SW voltammograms of all three mechanisms are given elsewhere [4, 16]. The symbol **Y** in schemes (2-3) refers to the electrochemically inactive substrate that is present in a large excess. Since the surface concentration of **Y** is constant in the course of the experiment, the chemical step in

reaction mechanisms (2-3) is assumed to be pseudo-first order in terms of the chemical kinetics.

In the simulation of the voltammetric outcome, the reduction current is assigned to be positive, while the oxidation current is negative. The simulated data are presented in the form of a dimensionless current defined as $\Psi = I/(nFSf\Gamma^*)$. Here, I is the symbol of the electric current, n is the stoichiometric number of electrons, S is the surface area of the working electrode, f is the SW frequency, and Γ^* is the initial surface concentration of Ox(ads) species. The frequency of SWV is defined as $f = 1/(2t_p)$, where t_p is the duration of a single potential pulse [4].

In all mechanisms, the features of simulated voltammograms are a function of a dimensionless electrode kinetic parameter $K_{\text{ET}} = k_s^\ominus/f$ that links the surface standard rate constant of the electron transfer k_s^\ominus (s^{-1}) with the SW frequency f . In addition, for both $C_{\text{rev}}E$ and EC_{rev} mechanisms (2-3), the voltammetric features depend on a dimensionless chemical kinetic parameter defined as $K_{\text{chem}} = k_c/f$. In the last equation, k_c is the cumulative rate constant of the chemical step, defined as $k_c = (k_f + k_b)$, where k_f and k_b are the rate constants of the forward and the backward chemical reactions, respectively. The physical meaning of k_c represents the rate at which the reversible chemical reaction can reach the equilibrium state, while the dimensionless parameter K_{chem} reflects the kinetics of the reaction relative to the time-frame of SWV. Furthermore, the voltammetric patterns of both $C_{\text{rev}}E$ and EC_{rev} mechanisms are affected by the equilibrium constant of the chemical reaction, which is defined as $K_{\text{eq}} = k_f/k_b$, representing the state of the established chemical equilibrium.

If not otherwise stated, the parameters of applied potential were set to the following values: SW amplitude $E_{\text{sw}} = 50$ mV, step potential $dE = 4$ mV, and frequency $f = 10$ Hz, while the electron transfer coefficient was set to $\alpha = 0.5$. More details of the algorithms used can be found in [4, 7, 8, 16]. The commercial software package MATHCAD 14 was used for calculation of all theoretical voltammograms.

3. RESULTS AND DISCUSSION

3.1. A short overview of some relevant voltammetric methods for evaluation of the rate of the electron transfer

Cyclic voltammetry is often explored as a reliable tool to get insights into the mechanism of

various electrochemical systems [1–3]. Moreover, a plethora of methods with CV have been reported to access the relevant kinetic data, as well [1, 2]. The methods of Nicholson [17], Gileadi [18], Kochi [19], Saveant [20] and Laviron [21] rely on the scan rate analysis of either the peak-to-peak potential separation or the mid-peak potential of cyclic voltammograms. For a simple electrode reaction of a dissolved redox couple, SWV also enables determination of the kinetics of the heterogeneous electron transfer based on the frequency analysis of the net-peak potential [4]. In addition, several alternative SWV methods allow access to the kinetic parameters of the heterogeneous electron transfer [4, 8, 22].

When the surface electrode mechanisms are concerned, SWV offers a variety of approaches to determine electrode kinetic parameters [4, 7, 8, 23–31]. Next to the net-peak potential vs. frequency analysis [4, 20], a plethora of methods have been developed based on the analysis of the net-peak current (Fig. S1) and the potential separation between split net-peak (Fig. S2 and S3) as a function of the frequency [4, 8, 16]. Moreover, kinetic analysis can be conducted by varying the SW amplitude at a constant SW frequency, i.e., at the constant scan rate of the voltammetric experiment [4, 27]. Relevant methods for accessing the kinetics of electron transfer reactions in SWV at constant scan rate are reported in [29–31]. These methods rely on amplitude-based quasi-reversible maximum [29] or kinetic evaluations at constant scan rate that are evaluated from the peak potentials of forward and backward currents as a function of the SW amplitude [30, 31].

3.2. Implications of the time analysis for surface $C_{rev}E$ and EC_{rev} mechanisms in SWV

When the time analysis is performed to estimate kinetic parameters of electrochemical systems coupled with preceding or follow-up chemical reactions, the situation gets far more complicated. It is worth emphasizing that for a simple surface electrode reaction an increase of the kinetic parameter of the electron transfer step K_{ET} is commonly followed by a shift of the net-peak potential to more positive values for an overall reductive electrode mechanism. For the surface $C_{rev}E$ mechanism, an increase of the chemical parameter K_{chem} causes a shift of the net-peak potential to more negative values, assuming a “constant value” of K_{ET} , as reported in [16, 25]. From the definition of both K_{ET} and K_{chem} ($K_{ET} = k_s^\circ/f$ and $K_{chem} = k_c/f$), it follows that the SW frequency affects these param-

eters equally. However, the two dimensionless parameters exhibit an effect opposite to the net-peak potential ($E_{net,p}$). Therefore, one expects that the frequency analysis will cause complex voltammetric features, which might be additionally affected by the magnitude of the equilibrium constant K_{eq} .

Several theoretical curves referring to $E_{net,p}$ vs. $\log(1/f)$ function are shown in Figure 1. These were calculated by assuming a constant value of $K_{chem} = 100$ for three different values of the equilibrium constant of the preceding chemical reaction. This analysis reveals the partial influence of the frequency which is manifested through the electrode kinetic parameter only.

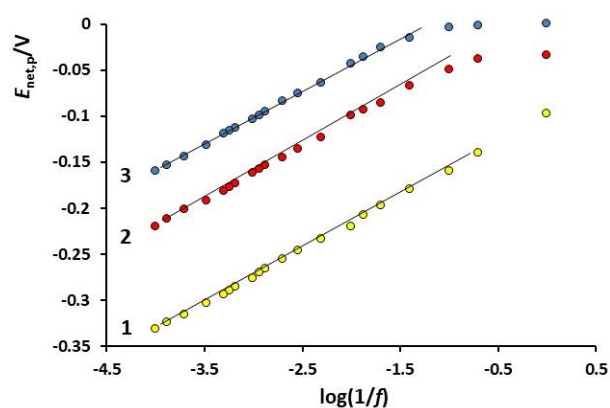
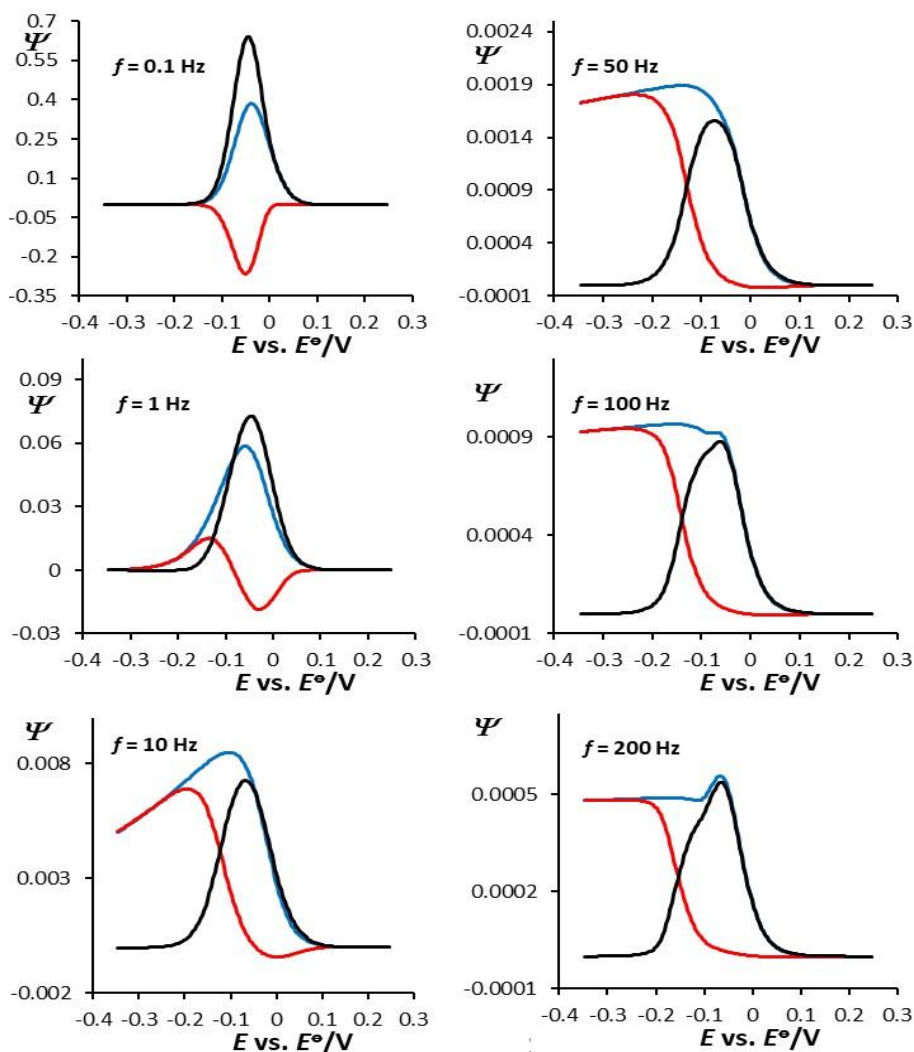


Fig. 1. Surface $C_{rev}E$ mechanism

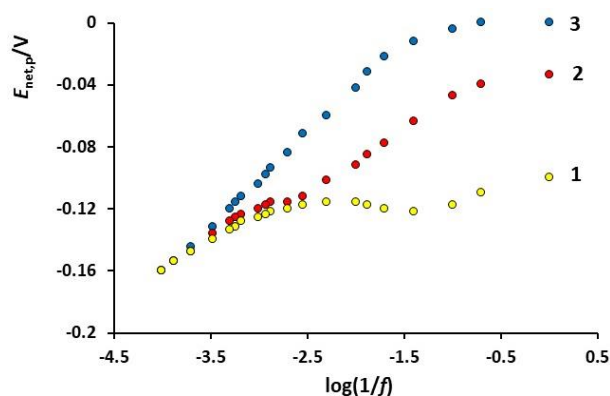
The net-peak potential $E_{net,p}$ as a function of the logarithm of the inverse SW frequency. Voltammetric curves are simulated by assuming a constant value of the dimensionless chemical rate parameter $K_{chem} = 100$. The other simulation conditions were: standard rate constant $k_s^\circ = 0.794 \text{ s}^{-1}$, electron transfer coefficient $\alpha = 0.5$, SW amplitude $E_{sw} = 50 \text{ mV}$, step potential $dE = 4 \text{ mV}$, temperature $T = 298 \text{ K}$, and the stoichiometric number of electrons $n = 2$. The values of the equilibrium constant of the preceding chemical reaction are $K_{eq} = 0.001$ (1), 0.1 (2), and 100 (3).

However, when an electrode mechanism is studied in a real experiment, the frequency simultaneously affects both kinetic parameters K_{ET} and K_{chem} . Hence, the frequency might produce rather complex voltammetric outcomes, as shown by exemplary voltammetric patterns, Fig. 2, calculated for a typical quasi-reversible electrode reaction. For $K_{eq} \leq 10^{-3}$, one observes almost a constant position of the net-peak by increasing the frequency (Fig. 2), while the forward and backward voltammetric components exhibit typical evolution of a quasi-reversible reaction under increasing frequency [4, 25].

Fig. 2. Surface $C_{rev}E$ mechanism

Evolution of the SW voltammetric patterns by increasing the SW frequency (the values are given in the plot) for the standard rate constant $k_s^\ominus = 0.794 \text{ s}^{-1}$, equilibrium constant $K_{eq} = 0.001$, and the cumulative chemical rate constant $k_c = 100 \text{ s}^{-1}$. Other conditions of the simulations are identical as for Fig. 1. In all voltammograms, the forward (reduction), backward (oxidation), and net current are depicted with blue, red, and black colors, respectively.

Shown in Figure 3 are the dependences between the net-peak potential $E_{net,p}$ vs. $\log(1/f)$, simulated for $k_s^\ominus = 0.794 \text{ s}^{-1}$, $k_c = 100 \text{ s}^{-1}$ and for three values of K_{eq} . The complex functional dependence of $E_{net,p}$ vs. $\log(1/f)$ is a consequence of the simultaneous variation of K_{ET} and K_{chem} by the SW frequency. These findings imply that the $E_{net,p}$ vs. f function can hardly be exploited to estimate k_s^\ominus or k_c of a surface $C_{rev}E$ system. The same holds true for the EC_{rev} mechanism (cf. Fig. 4).

Fig. 3. Surface $C_{rev}E$ mechanism

Dependence of the net-peak potential $E_{net,p}$ on the logarithm of the inverse SW frequency. The simulation conditions are as follows: standard rate constant $k_s^\ominus = 0.794 \text{ s}^{-1}$, cumulative chemical rate constant $k_c = 100 \text{ s}^{-1}$, SW amplitude $E_{sw} = 50 \text{ mV}$, and step potential $dE = 4 \text{ mV}$. The values of the

equilibrium constant are $K_{\text{eq}} = 0.001$ (1); 0.1 (2) and 100 (3). Other conditions of the simulations are identical as for Figure 1.

As elaborated in [4], a simple and efficient tool to measure the kinetics of the electron transfer of a simple surface electrode mechanism is the feature named “quasi-reversible maximum” (QRM), which is based on the analysis of the net-peak current. The parabolic dependence of the net-peak current as a function of $\log(1/f)$ can provide access to the standard rate constant of the electron transfer in a very simple manner [4], provided the electron transfer coefficient α is previously determined. The last parameter (α) can be evaluated from the half-peak width of square-wave voltammograms vs temperature dependence, performed in the regions of very slow electron transfer (i.e. at large frequencies) and following the methodology elaborated in [22]. Alternatively, the electron transfer coefficient α can also be evaluated from the features of split SW peaks at fast electrode reactions, as explained in [4].

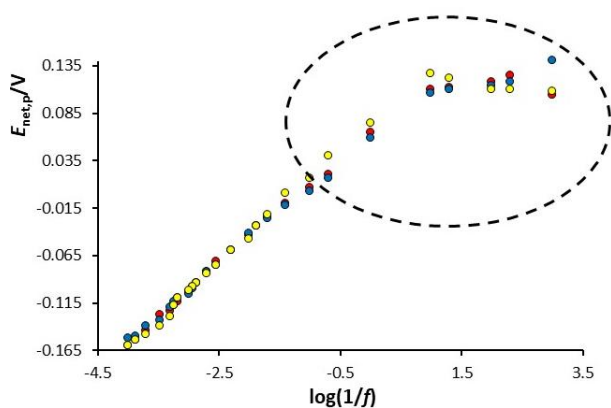


Fig. 4. Surface EC_{rev} mechanism

Dependence of the net-peak potential $E_{\text{net,p}}$ on the logarithm of the inverse SW frequency. The simulation conditions are as follows: standard rate constant $k_s^\ominus = 0.794 \text{ s}^{-1}$, cumulative chemical rate constant $k_c = 1 \text{ s}^{-1}$, SW amplitude $E_{\text{sw}} = 50 \text{ mV}$, and step potential $dE = 4 \text{ mV}$. The values of the equilibrium constant are $K_{\text{eq}} = 0.001$ (red circles); 1 (blue circles), and 100 (yellow circles). Other conditions of the simulations are identical to those described in Figure 1.

The evolution of QRM as a function of chemical kinetics, simulated for the surface $\text{C}_{\text{rev}}\text{E}$ mechanism, is depicted in Fig. 5. In a broad context, the position of the QRM is sensitive to multiple parameters, including the SW amplitude (E_{sw}), step potential (dE), electron transfer coefficient (α), and the equilibrium constant (K_{eq}). Comprehensive simulations revealed that the QRM emerg-

es for the $\text{C}_{\text{rev}}\text{E}$ mechanism if the preceding chemical reaction is associated with $K_{\text{eq}} > 0.01$ and $K_{\text{chem}} > 0.05$. It is important to point out that for the conditions of Fig. 5 the position of the QRM is virtually independent of the chemical rate parameter k_c . Furthermore, in a broad interval of k_c values, the position of the QRM is slightly dependent on K_{eq} , as shown in Fig. 6. Hence, the data presented in Figs. 5 and 6 imply that the QRM is a suitable feature for estimating the standard rate constant of the electron transfer (k_s^\ominus), in spite of the fact that the kinetic and thermodynamic parameters of the preceding chemical reaction of the $\text{C}_{\text{rev}}\text{E}$ mechanism are not exactly known, which is valid for $\log(K_{\text{eq}}) \geq -2$ and for large range of k_c values.

For the surface EC_{rev} mechanism, however, quite a different evolution of the QRM was observed for different values of k_c , particularly in the region of moderate-to-low values of K_{eq} [16, 28]. An increase of the chemical rate parameter k_c (roughly, within the region $0.1 \text{ s}^{-1} < k_c < 10 \text{ s}^{-1}$) affects not only the position but also the shape of the QRM (Fig. 7). In some regions of SW frequency, one observes so-called “local maximums” which appear due to the specific interplay of k_s^\ominus , k_c , and K_{eq} . These findings suggest that the rate of the chemical step might significantly influence the rate of the electron transfer step for the EC_{rev} system, especially those featuring moderate and low values of the equilibrium constant.

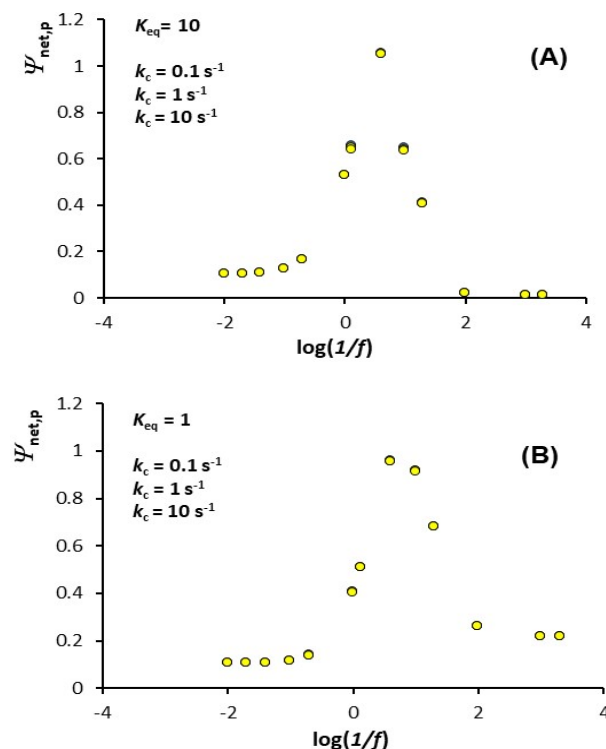


Fig. 5. Surface $\text{C}_{\text{rev}}\text{E}$ mechanism

Dependence of the net-peak current $\Psi_{\text{net,p}}$ on the logarithm of the inverse SW frequency, simulated for different values of the cumulative chemical rate constant k_c . The simulation conditions are as follows: standard rate constant $k_s^\ominus = 0.1 \text{ s}^{-1}$, step potential $dE = 4 \text{ mV}$, and the equilibrium constants are $K_{\text{eq}} = 10$ (for panel **A**) and 1 (for panel **B**). Other conditions of the simulations are identical to those described in Figure 1.

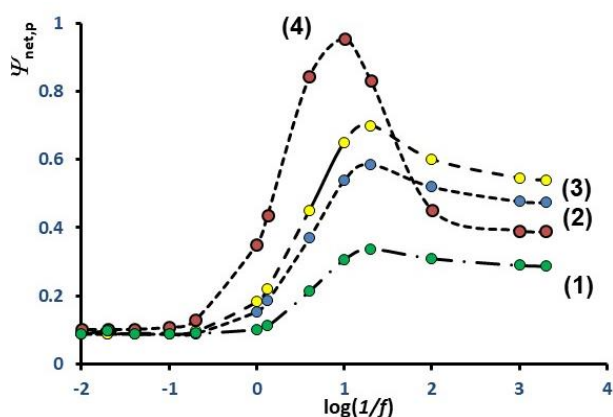


Fig. 6. Surface $C_{\text{rev}}E$ mechanism

Dependence of the net-peak current $\Psi_{\text{net,p}}$ on the logarithm of the inverse SW frequency, calculated for different values of the equilibrium constant: $K_{\text{eq}} = 0.03$ (1); 0.07 (2); 0.1 (3) and 0.56 (4). The simulation conditions are: standard rate constant $k_s^\ominus = 0.1 \text{ s}^{-1}$ and the cumulative chemical rate constant $k_c = 1 \text{ s}^{-1}$. Other conditions of the simulations are identical to those described in Fig. 1.

The reasons for this complex behavior associated with the complex evolution of the backward component of the SW voltammogram are partly elaborated elsewhere [28, 32]. The features of the QRM of the surface EC_{rev} mechanism are a consequence of the specific chronoamperometric features of this mechanism. They are significantly affected by the proceeding of the follow-up chemical reaction within the so-called “dead time” of potential pulses, where the current is not measured [28, 32]. The phenomenon depicted in Figure 7 is quite different from the surface $C_{\text{rev}}E$ mechanism. It implies that the kinetic analysis by varying the SW frequency (i.e. time analysis) for the surface EC_{rev} mechanism by means of the QRM is not straightforward. In addition, the effect of the equilibrium constant on the position and morphology of the QRM is rather complex, as illustrated in Fig. 8. In previous studies [16, 28], it has been elaborated that the feature known as “split net SW peak” of the surface $C_{\text{rev}}E$ and EC_{rev} mechanisms is also

sensitive to the value of K_{chem} and K_{eq} . Consequently, the estimation of the standard rate constant based on the split net SW peak must be taken with a caution, when the analysis is done by altering the SW frequency, for both $C_{\text{rev}}E$ and EC_{rev} mechanisms. However, it is worth mentioning that the analysis of the splitting of net-peak by altering the substrate concentration can be effectively explored as a diagnostic criterion to characterize both surface EC_{rev} and $C_{\text{rev}}E$ mechanisms featuring very fast kinetics of the electron transfer step [16].

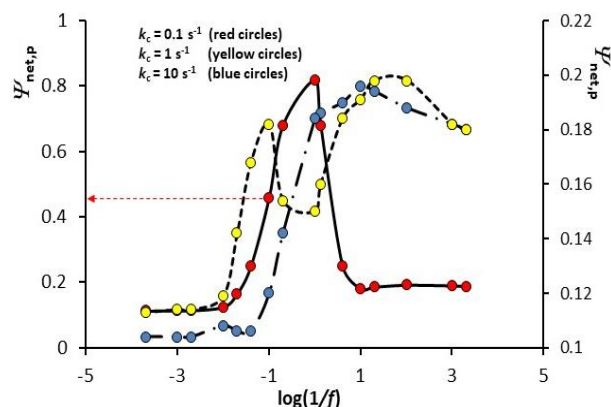


Fig. 7. Surface EC_{rev} mechanism

Dependence of the net-peak current $\Psi_{\text{net,p}}$ on the logarithm of the inverse SW frequency, calculated for different values of the cumulative chemical rate constant k_c (the values of k_c are given on the plot); the equilibrium constant is $K_{\text{eq}} = 0.01$, and the standard rate constant is $k_s^\ominus = 10 \text{ s}^{-1}$. The curves with yellow and blue circles refer to the right ordinate. Other conditions of the simulations are identical to those described in Figure 1.

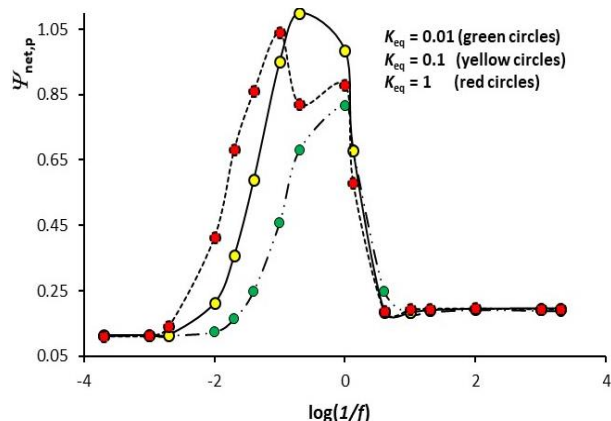


Fig. 8. Surface EC_{rev} mechanism

Dependence of the net-peak current $\Psi_{\text{net,p}}$ on the logarithm of the inverse SW frequency for dif-

ferent values of the equilibrium constant K_{eq} given on the plot. The standard rate constant is $k_s^\ominus = 10 \text{ s}^{-1}$ and the cumulative chemical rate constant is $k_c = 0.1 \text{ s}^{-1}$. Other conditions of the simulations are identical to those described in Figure 1.

4. CONCLUSIONS

Although the surface electrode mechanisms coupled with either preceding or following chemical reactions are well elaborated under conditions of SWV [4, 8, 15, 16, 25, 28, 32], some aspects of these systems need to be taken into critical consideration. Many theoretical voltammetric methods that were developed to get access to the kinetics of a wide diversity of redox systems coupled with chemical reactions rely on the time-analysis of some relevant voltammetric parameters [1–3, 20, 21]. If the time-analysis is performed to get an access to the kinetics of electrode reactions associated with chemical equilibria in SWV, caution must be taken about the methodology used. For the surface $\text{C}_{\text{rev}}\text{E}$ mechanism, the quasi-reversible maximum based on the frequency variation can be successfully explored for estimation of the standard rate constant k_s^\ominus without previous knowledge of the kinetics and thermodynamics of the chemical reaction, in a broad interval of values for both k_c and K_{eq} . This is not the case, however, for the surface EC_{rev} mechanism, where the kinetics of the electrode reaction can be significantly affected by the kinetics of the follow-up chemical step. Minimization of some of the effects caused by the rate of the follow up chemical reaction can be eventually avoided by performing experiments at frequencies larger than 100 Hz, preferably between 100 Hz and 500 Hz. Alternatively, methods based on the constant time of the experiment (i.e., constant scan rate of the voltammetry) are recommended [29, 30] to access the kinetics of electrode reaction by using square-wave amplitudes between 25 mV and 100 mV. It is important to stress that the phenomenon of net-peak splitting might be significantly affected by the kinetics of the chemical reaction for both $\text{C}_{\text{rev}}\text{E}$ and EC_{rev} mechanisms. This phenomenon, however, can be explored as a simple tool for qualitative differentiation between $\text{C}_{\text{rev}}\text{E}$ and EC_{rev} mechanisms [16]. In the end, it is worth mentioning that the need of a theoretical study, such as the one presented in this work, has been implicitly addressed in two recent theoretical papers by Molina, Compton et al. [34, 35].

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