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# THE NET PEAK SPLITTING PHENOMENON IN SQUARE-WAVE VOLTAMMETRY: A SIMPLE DIAGNOSTIC TOOL TO DISTINGUISH BETWEEN SURFACE ELECTRODE MECHANISMS ASSOCIATED WITH DIFFERENT CHEMICAL REACTIONS

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Utilizing pulse voltammetric techniques for the electrochemical analysis of lipophilic redox systems has proven to be an effective method for studying a diverse range of redox compounds, from simple molecules to intricate proteins. To extract relevant thermodynamic and kinetic data from electrochemical analysis of redox systems exhibiting strong surface activity, there's a crucial need to decipher the underlying electrochemical mechanism in the system being examined. The "split net peak" phenomenon, a defining characteristic observed in all surface-active redox systems featuring fast electron transfer under square-wave voltammetry conditions, has been investigated in this study to establish diagnostic criteria for identifying surface electrochemical mechanisms associated with preceding, subsequent, regenerative, and reactant-inactivating chemical reactions. This understanding can be achieved by tracking the influence of the chemical reaction rate on the split square-wave voltammetric peaks in a particular electrochemical mechanism. The approach reported in the current work enables a very simple and precise differentiation between the elaborated mechanisms frequently encountered in protein-film voltammetry methodologies.

**Keywords:** surface CE mechanism; surface EC mechanism; surface EC′ regenerative mechanism; kinetics of electron transfer; split square-wave voltammograms

## ФЕНОМЕН НА РАЗДВОЈУВАЊЕ НА РЕЗУЛТАНТНИОТ ПИК ВО КВАДРАТНО-БРАНОВА ВОЛТАМЕТРИЈА: ЕДНОСТАВЕН ДИЈАГНОСТИЧКИ КРИТЕРИУМ ЗА РАЗЛИКУВАЊЕ ПОВРШИНСКИ ЕЛЕКТРОДНИ МЕХАНИЗМИ ШТО СЕ ПОВРЗАНИ СО РАЗЛИЧНИ ХЕМИСКИ РЕАКЦИИ

Примената на пулсните волтаметриски техники во електрохемиските анализи на липофилни редокс-системи се смета за добар експериментален приод кон електрохемиското студирање на различни редокс-аналити кои можат да бидат едноставни молекули, но и комплексни редокс-ензими и протеини. За да се евалуираат релевантни термодинамички и кинетички параметри од електрохемиските анализи применети на редокс-системи што покажуваат силна површинска активност, од суштинска важност е да се знае електрохемискиот механизам според кој испитуваниот систем претрпува електрохемиска трансформација. Феноменот „раздвојување на резултантниот пик“ е специфична карактеристика кај сите површински електродни механизми што се карактеризираат со многу брз чекор на размена на електрони помеѓу работната електрода и молекулите од формите на површински активниот аналит. Во рамките на овој труд, овој феномен на раздвојување на резултантниот пик е студиран со примена на квадратно-бранова волтаметрија, при што неговите својства се употребени за утврдување дијагностички критериуми што можат да послужат при идентификација на површински електродни механизми кои се поврзани со претходна хемиска реакција, последователна хемиска реакција, регенеративна хемиска реакција и со хемиска реакција на деактивација на почетниот реактант. Притоа релевантни податоци за природата на хемиските реакции можат да се добијат со едноставен методолошки приод, преку студирање на влијанието на хемиските кинетички параметри врз феноменот на раздвојување на резултантниот пик кај соодветните електродни механизми. Методологијата претставена во овој труд е едноставен приод што овозможува брзо и едноставно препознавање и разликување на студираните механизми што многу често се среќаваат во техниката на волтаметрија на протеински филм.

**Клучни зборови:** површински CE механизам; површински EC механизам; површински EC′ регенеративен механизам; кинетика на пренос на електрони; раздвоен резултантен квадратно-бранов волтамограм.

## 1. INTRODUCTION

When searching for efficient, rapid, and reliable methods in the electrochemical analysis of so-called "redox molecules", voltammetric techniques emerge as a leading choice due to their adeptness at analyzing a spectrum of redox systems ranging from simple inorganic ions to complex enzymes and proteins. Square wave voltammetry (SWV) is arguably the most advanced technique within the family of pulse voltammetric methods, which are viewed as the pinnacle of electrochemical instruments.[1,2] Characterized by unique properties with respect to the current sampling procedure, SWV has emerged as a leading voltammetric technique both for mechanistic characterizations and kinetic measurements of various redox systems.[1] The distinctive characteristics of SWV as a voltammetric technique arise mainly from its pulsed potential waveform that is synchronized with a coordinated current measurement approach. This combination effectively minimizes major interferences caused by undesired non-Faradaic processes often going on at the electrode-electrolyte interface.[1–4] In the general context, SWV superimposes square wave potential pulse waveforms on a permanently raising staircase ramp. The customary current sampling protocol in SWV focuses on the latter time segment of each applied potential pulse, in which the contribution of so-called "charging currents" is effectively minimized, while the Faradaic currents (occurring due to electron exchange between the working electrode and the redox system in consideration) still have significant magnitude.[1–3] While the currents detected at the end of direct pulses are referred to as "forward" current components, the currents measured in reverse pulses represent the "reverse" (or backward) current components (bearing a sign opposite to that of the forward currents) of square-wave voltammetric outputs. In SWV, the so-called "net" current-voltage curve is derived from the difference between the forward and reverse currents. Essentially, this represents a cumulative measure of the magnitudes of both forward and backward current components at each applied potential.[1,3] As reported in,[1] the SWV proves invaluable in gauging surface-confined redox systems, thus serving as an efficient tool, especially in the protein-film voltammetry methodology.[5–8] As underscored in several significant books and theoretical research papers,[1,9–15] numerous phenomena can be observed in the SW voltammetric data, which relate to diverse electrochemical mechanisms associated with the pronounced surface activity of all entities involved in the electrode mechanism. Notably, the "quasi-reversible maximum"[1] and the "splitting of the net SW peak"[1,14] are perceived as distinctive hallmarks of redox systems showing strong surface activity of the analyte's redox active molecules at the working electrode. While both mentioned phenomena help in determining kinetic parameters related to the electron transfer rate through elegant protocols,[1] the ability of the "split net SW peak" phenomenon to distinguish between electrochemical mechanisms coupled with chemical reactions has not been sufficiently explored. In this study, we delve into the "splitting of net SWV peak" phenomenon to develop straightforward diagnostic protocols that can differentiate between surface electrochemical mechanisms linked to different types of chemical reactions.

## 2. EXPERIMENTAL

### 2.1. *Reactions schemes of electrochemical mechanisms considered*

The electrode mechanisms elaborated in this work are as follows:

1. "Simple" surface electrode mechanism of a strongly adsorbed redox couple (E-mechanism):

$$\text{E:}\qquad \text{Ox(ads)} + \text{e-} \overset{K_s^{\ominus},\ \alpha}{\rightleftarrows} \text{Red(ads)} \qquad (1)$$

2. Surface electrode mechanism of a strongly adsorbed redox couple in which the product of electrode transformation is involved in reversible follow-up chemical reaction (surface ECrev mechanism, where "C" denotes chemical reaction):

$$\begin{array}{lll}\text{E:} & \text{Ox(ads)} + \text{e-} \xrightleftharpoons{K_s^{\ominus},\ \alpha} & \text{Red(ads)} \\ \text{Crev:} & \text{Red(ads)} + \text{Y} \xrightleftharpoons[K_b]{K_f} & \text{Z(ads)}\end{array} \qquad (2)$$

3. Surface electrode mechanism of a strongly adsorbed redox couple, where the product of electrode transformation is involved in an irreversible chemical reaction in which the initial electrochemically active reactant is regenerated (surface EC' regenerative mechanism):

$$\begin{array}{lll}\text{E:} & \text{Ox(ads)} + \text{e-} \xrightleftharpoons{K_s^{\ominus},\ \alpha} & \text{Red(ads)} \\ \text{C:} & \text{Red(ads)} + \text{Y} \xrightarrow{K_c} & \text{Ox(ads)}\end{array} \qquad (3)$$

4. Surface electrode mechanism of a strongly adsorbed redox couple in which the reactant of the electrode transformation is obtained via a preceding reversible chemical reaction (surface CrevE mechanism):

$$\begin{array}{lll}\text{Crev:} & \text{A(ads)} + \text{Y} \xrightleftharpoons[K_b]{K_f} & \text{Ox(ads)} \\ \text{E:} & \text{Ox(ads)} + \text{e-} \xrightleftharpoons{K_s^{\ominus},\ \alpha} & \text{Red(ads)}\end{array} \qquad (4)$$

5. Surface electrode mechanism of a strongly adsorbed redox couple in which the initial electrochemically active reactant (Ox) is also involved in a parallel irreversible chemical reaction of inactivation:

$$\begin{tikzcd}\text{E:} & \text{Ox(ads)} + \text{e-} \arrow[r, leftrightarrow, "K_s^{\ominus},\ \alpha"] \arrow[d] & \text{Red(ads)} \\ \text{C:} & +\text{Y} \arrow[r, "K_c"] & \text{Z(ads)}\end{tikzcd} \qquad (5)$$

### 2.2. *Definition of major kinetic and thermodynamic parameters affecting the voltammetric outputs of considered surface electrochemical mechanisms*

In all models, the elaborated theoretical electrochemical mechanisms (1–5) are considered under certain assumptions. All models posit that the molecules of all species involved (A, Ox, Red, and Z) are firmly anchored to the electrode surface while neglecting any further mass transport during the voltammetric experiment. It is also presupposed that the molecules of adsorbed species form a monomolecular layer on the working electrode surface without any kind of interactions among the molecules of immobilized electroactive components. The concentration of chemical agent "Y" is supposed to be present in excess, so all chemical steps in mechanisms (2) to (5) can be defined to be of pseudo-first order. The initial amount of Ox(ads) species available for electrode transformation in CrevE mechanism (4) and the equilibrium amount of Red(ads) in the surface ECrev mechanism (2) are a function of the equilibrium constant of the chemical reaction $K_{\mathrm{eq}}$, defined equally in both cases as $K_{\mathrm{eq}} = k_f/k_{\mathrm{b}}$. The rate of chemical resupply of Red species in mechanism (2) and Ox species in electrode mechanisms (4) during the voltammetric experiment is governed by identical the chemical dimensionless rate parameter $K_{\mathrm{chem}}$, defined as $K_{\mathrm{chem}} = (k_{\mathrm{f}} + k_{\mathrm{b}})/f$. In the last two expressions, $k_{\mathrm{f}}$ and $k_{\mathrm{b}}$ are rate constants of direct and reverse chemical reactions, respectively. For electrochemical mechanisms (3) and (5), the dimensionless chemical rate parameter is defined as $K_{\mathrm{chem}} = k_{\mathrm{c}}/f$, where $k_{\mathrm{c}}$ is the rate constant of the irreversible chemical reaction in (5) or the irreversible regenerative chemical reaction in (3). Since the chemical kinetics of mechanisms (2) to (5) are of pseudo-first order, the magnitudes of $K_{\mathrm{chem}}$ in all models (2) to (5) are a function of the substrate concentration of "Y" $c$(Y). In all models elaborated (1–5), the rate of the electron transfer between the working electrode and the redox active species Ox and Red is represented via the dimensionless kinetic parameter $K_{\mathrm{ET}}$ defined by the expression $K_{\mathrm{ET}} = k_{\mathrm{s}}^{\ominus}/f$. In the last expression, $k_{\mathrm{s}}^{\ominus}$ ($\mathrm{s}^{-1}$) is the standard rate constant of the electron transfer step, while $f$ is the SW frequency of applied pulses. A detailed description of all theoretical models considered in this work is given in[1, 9–16].

## 3. RESULTS AND DISCUSSION

A distinct hallmark of all electrochemical mechanisms exhibiting adsorption phenomena of the molecules of electrochemically active species is the "splitting of the net peak", which is an event taking place when such redox systems are analyzed with square-wave voltammetry as a working technique.[1,14] The phenomenon of substantial separation between cathodic and anodic peaks of lipophilic redox systems, whose molecules are strongly confined at the working electrode surface, stems from the current sampling protocol used in SWV.[1] This can be further complicated by the occurrence of various chemical reactions in which the surface-confined redox molecules might be involved.[9–13]

Bearing in mind the current measuring protocol in SWV,[1] the application of each potential pulse disrupts the established equilibrium between Ox(ads) and Red(ads) at the electrode surface, thus prompting a flow of current that tends to restore this equilibrium. Achieving the new equilibrium requires additional energy, leading to a shift in both the cathodic and anodic peaks towards opposite directions. This sequence of events results in net SW peak splitting as a final output of redox transformations taking place both in the "dead-time" (i.e., non-current measuring time) and in the current-measuring segments of the applied potential pulses (Fig. 1).

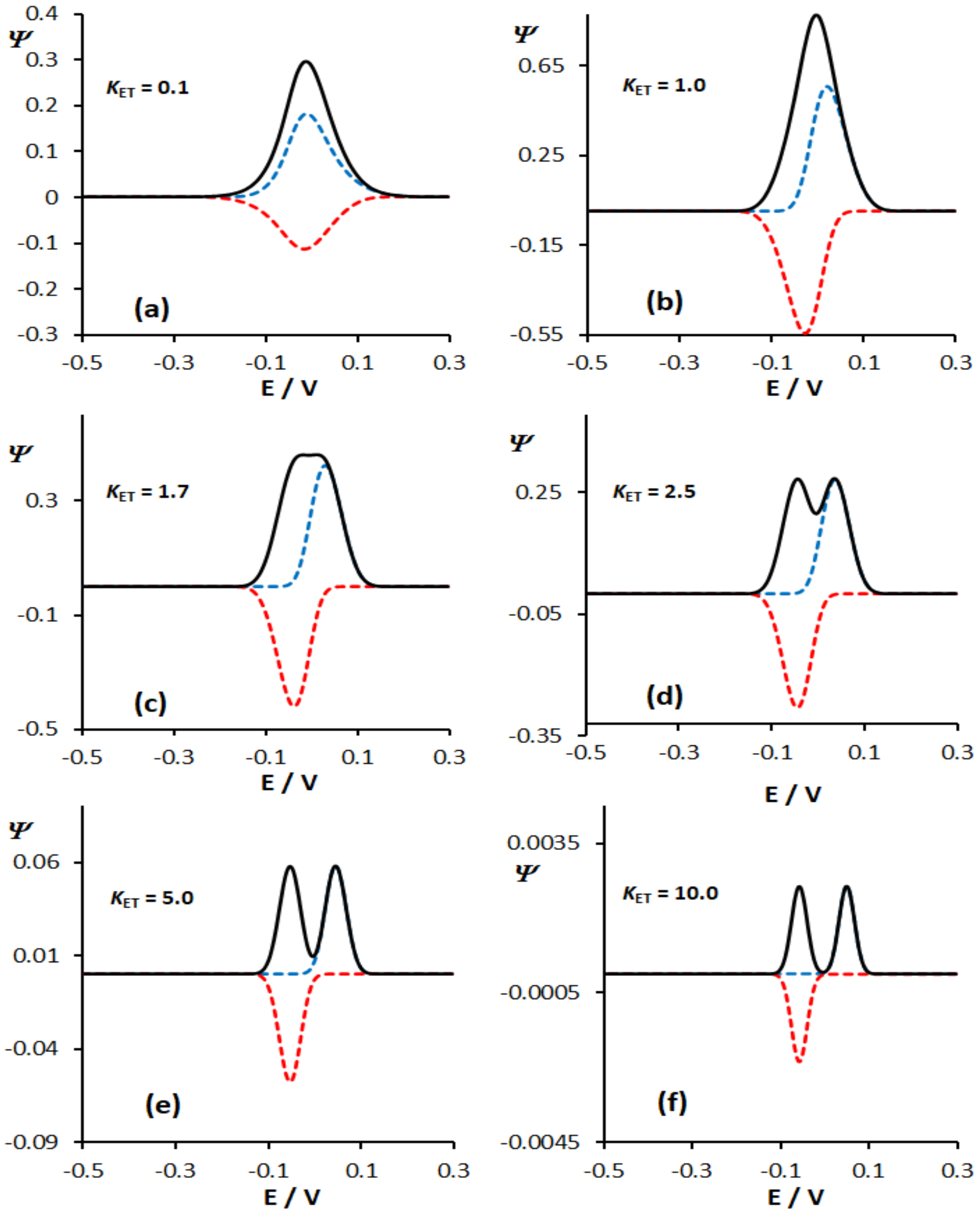

**Fig. 1.** Square-wave voltammograms of "simple" Ox(ads) + $1e^-$ = Red(ads) surface electrochemical mechanism (1) calculated as a function of dimensionless rate parameter of electron transfer step. The other simulation conditions were as follows: square wave amplitude $E_{sw}$ = 60 mV, potential step d$E$ = 4 mV, electron transfer coefficient $\alpha$ = 0.5, number of exchanged electrons $n$ = 1, thermodynamic temperature $T$ = 298 K, starting potential $E_s$ = 0.30 V (vs. the formal redox potential of Ox/Red couple that was defined to be 0.00 V). In all mechanisms, the blue color is associated with the forward (reduction) currents, while the red color is linked to the backward (reoxidation) currents. The net SWV currents are associated with black colors. The magnitudes of $K_{ET}$ are given in the charts.

In,[14] it is comprehensively elaborated that the greater the rate of electron transfer, the more pronounced the separation of the reduction and oxidation peaks will be. If the redox equilibrium fully reestablishes before measuring the current at the end of applied SW pulses, then only a minimal amount of the redox molecules Ox(ads) and Red(ads) remains, thus producing a very slight detectable current in the SW pulses' current measuring segment. An example of splitting the net SW peak of a "simple" surface electrode mechanism as a function of the dimensionless rate parameter of electron transfer, $K_{\mathrm{ET}}$, is presented in the voltammetric patterns in Figure 1. As reported in,[1,14] the net SW peak splitting phenomenon is influenced by both the rate of the electron transfer reaction and the magnitudes of parameters of the exciting signal, such as amplitude $E_{\mathrm{sw}}$ and potential step d$E$. By using the dependences between the potential separation of split SW peaks as a function of $K_{\mathrm{ET}}$, $E_{\mathrm{sw}}$, and d$E$ reported in,[14] the split peak phenomenon can be applied both for kinetic and thermodynamic evaluations related to the electron transfer step.

In voltammetry of surface-confined redox systems where a chemical reaction is associated with the electron transfer step, the splitting of the net SW peak is significantly influenced by the kinetics of the preceding, subsequent, regenerative, or parallel chemical process involved. In this study, we delve into this phenomenon to clearly differentiate between surface electrode mechanisms where diverse chemical reactions are linked to the electron transfer step (see reaction schemes 1–5). Relevant reports on theoretical voltammetric modeling related to the considered mechanisms (1–5) are available in[1,9–16.]

The influence of the dimensionless rate parameter of electron transfer $K_{\mathrm{ET}}$ on the theoretical SW voltammetric outputs of the "simple" surface mechanism (1) is already well-documented and thoroughly discussed in[1]. In brief, as $K_{\mathrm{ET}}$ increases, all current components also increase, peaking when $K_{\mathrm{ET}}$ equals a value of ≈ 1.0 (as seen in Figs. 1a–b). Beyond this, any further rise in $K_{\mathrm{ET}}$ results in a diminishment of all current components, accompanied by an expansion of the potential gap between the cathodic and anodic peaks. Specifically, at an $E_{\mathrm{sw}}$ of 60 mV and d$E$ of 4 mV, the net SW peak divides in the vicinity of a $K_{\mathrm{ET}}$ value of around 2.0 (as illustrated in Figs. 1c–d). Subsequent increases in $K_{\mathrm{ET}}$ are marked by a pronounced decrease in all SW voltammogram current components and a widening of the potential separation between the divided SW peaks (as depicted in Figs 1d–e). The underlying causes and specificities of these effects in the SW voltammograms of a "simple" surface electrochemical mechanism (1) are nicely detailed in the research conducted by Mirceski and Lovric.[14]

When evaluating electrochemical mechanisms coupled with chemical reactions, one might intuitively assume that the surface EC mechanism (2) should be straightforward to consider. This notion arises from the belief that the follow-up chemical step related to the Red(ads) species would "consume" the product of the electrochemical transformation, resulting in a consistent diminishment of the backward current (re-oxidation in this context) as the chemical reaction rate intensifies. Yet, as demonstrated in the voltammograms in Figure 2, this expectation doesn't align with the actual findings.

In scenarios with a fast electron transfer step ($K_{\mathrm{ET}} = 10$) and a relatively high value for the equilibrium constant of the subsequent chemical step ($K\mathrm{eq} > 5$), the impact of the rate of the follow-up chemical reaction on the features of the current components in theoretical voltammograms becomes quite intricate. In circumstances where the dimensionless chemical rate parameter $K_{\mathrm{chem}}$ is between 0.001 and 0.01, it's observed that an increase in $K_{\mathrm{chem}}$ results in a notable amplification (contrary to the expected decrease!) of the backward current component (refer to voltammograms 2a–c). The net SWV peak splitting phenomenon disappears when $K_{\mathrm{chem}}$ exceeds 0.05 (as shown in Fig. 2d). It's only at greater values of the chemical rate parameter, $K_{\mathrm{chem}}$, that the voltammograms begin to exhibit the anticipated shape for an ECrev mechanism, accompanied by a concomitant decrease in the backward current as $K_{\mathrm{chem}}$ rises. Moreover, the peak potential of the net SWV peak begins to shift to more positive potentials with the increase of $K_{\mathrm{chem}}$, which aligns with expectations for an EC mechanism.[1] As reported in[12,] the combined effects of the rate at which Red is resupplied through the chemical reaction and the quickly set electrochemical equilibrium between Ox and Red during the "dead-time" of potential pulses significantly contribute to the features at the voltammograms displayed in Figure 2. The specific voltammetric characteristics in Figure 2, obtained upon an increase in the magnitude of the chemical rate parameter, $K_{\mathrm{chem}}$, are uniquely representative of the surface ECrev mechanism featuring the net-peak splitting phenomenon in SWV.

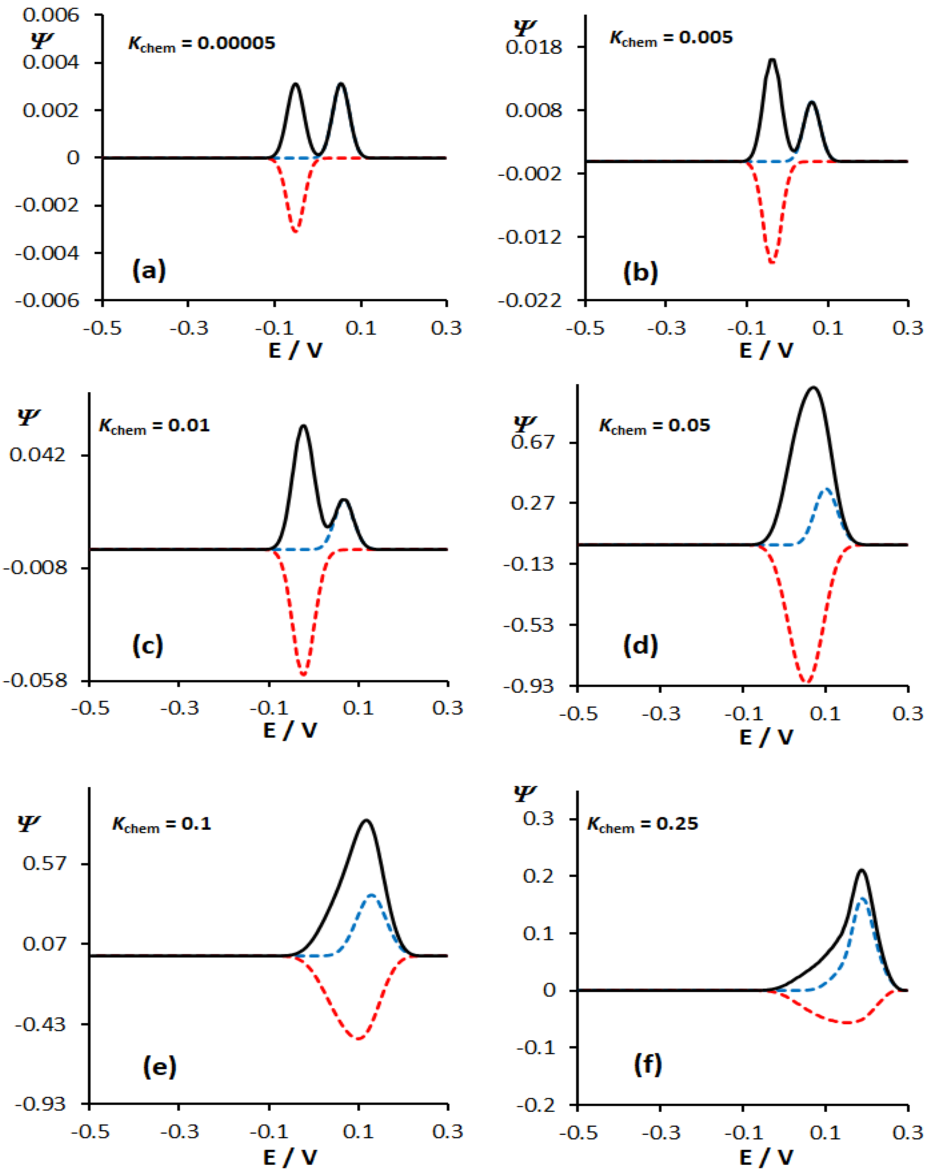

**Fig. 2.** Square-wave voltammograms of surface electrochemical Mechanism (2) associated with a follow-up reversible chemical step (surface ECrev mechanism) calculated as a function of a dimensionless chemical rate parameter $K_{chem}$. The value of the dimensionless rate parameter of electron transfer was set to $K_{ET} = 10$, while the equilibrium constant of chemical reaction was set to $Keq = 10$. The magnitudes of $K_{chem}$ used for this set of simulations are given in the charts. Other simulation conditions were the same as in Figure 1.

Regarding the impact of the chemical rate parameter in the *surface regenerative EC' mechanism (3)* characterized by a large value of $K_{ET}$, it's essential to highlight that $K_{chem}$ exhibits some distinct characteristics in this context. With an increase in the rate of the regenerative chemical reaction in the electrochemical Mechanism (3), a permanent decrease in the backward current component is seen, accompanied by a corresponding rise in the forward current component (as shown in Figs 3a–c). When $K_{chem}$ values exceed 0.01, the phenomenon of splitting the net SWV peak disappears (as illustrated in Fig. 3d), leading to the emergence of a single net SW voltammetric peak that intensifies with subsequent increases in $K_{chem}$. The "sigmoidal" profiles of the forward and backward current components in the SW voltammograms (Figs. 3e–f) become evident at high chemical resupply rates of Ox. These recognizable patterns (Figs. 3e–f) arise when the regenerative reaction rate is fast enough to convert chemically Red back to Ox within the current measurement duration of potential pulses.[9,15] The occurrences outlined in Figure 3 are characteristic of the surface regenerative EC' mechanism in SWV. Indeed, voltammetric patterns in Figure 3 can be employed for straightforward characterization of this mechanism under conditions where the net SWV peak splits.

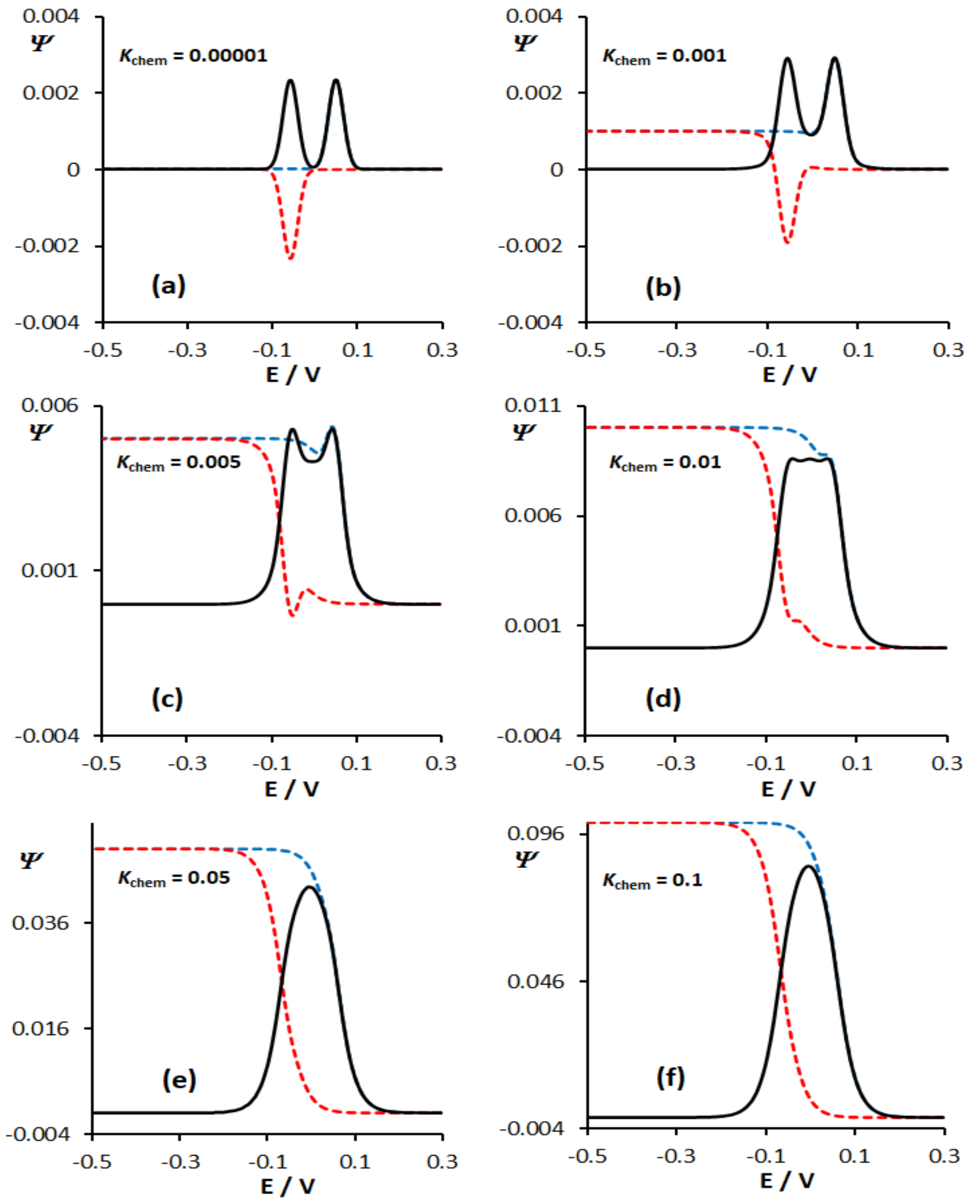

**Figure 3.** Square-wave voltammograms of surface electrochemical Mechanism (3) associated with regenerative chemical step (surface EC' regenerative mechanism) calculated as a function of dimensionless chemical rate parameter $K_{chem}$. The value of the dimensionless rate parameter of electron transfer was set to $K_{ET} = 10$. The magnitudes of $K_{chem}$ used for this set of simulations are given in the charts. Other simulation conditions were same as in Figure 1.

The *surface CE mechanism (4)* arguably demonstrates the most intricate behavior among all electrochemical mechanisms coupled with chemical reactions[10] considered in this work. When the chemical rate parameter exceeds the $K_{chem}$ magnitude of 0.01 (at $K$eq = 10), the splitting event vanishes, concurrently intensifying the forward current components, which assume somewhat unconventional forms (as depicted in Figs 4b–c). A continued increase in the magnitude of $K_{chem}$ around the magnitude of 0.5 results in a single net SWV peak that is dominated by a notably accentuated forward current component (illustrated in Fig. 4d). Ultimately, the net SW peak splitting event reappears at rather high rates of the chemical step ($K_{chem} >$ 30, as seen in Figs. 4e–f). The dynamic interplay between the rates of Ox production through the chemical reaction and the rate of Ox's electrochemical interconversion to Red, both during the "dead-time" and the current measurement portion of SW potential pulses, significantly influences the voltammetric patterns showcased in Figure 4. The attributes displayed in Figure 4, which emerge upon increasing the rate of the preceding chemical reaction, are emblematic of the surface CE mechanism. These traits contrast distinctly with those observed in the surface EC mechanism (2) and the surface regenerative EC' mechanism (3) considered in this work.

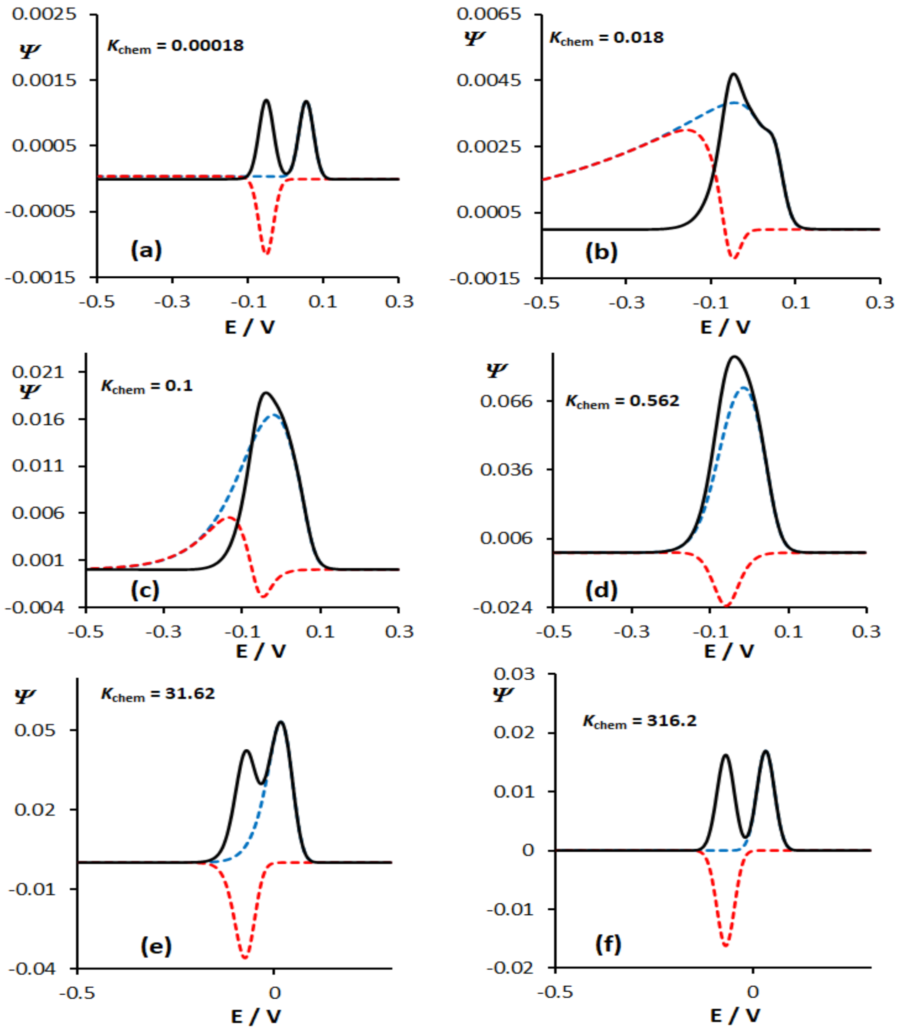

**Fig. 4.** Square-wave voltammograms of surface electrochemical mechanism (4) associated with following a reversible preceding chemical step (surface CrevE mechanism) calculated as a function of dimensionless chemical rate parameter $K_{\mathrm{chem}}$. The value of the dimensionless rate parameter of electron transfer was set to $K_{\mathrm{ET}} = 10$, while the equilibrium constant of chemical reaction was set to $K\mathrm{eq} = 10$. The magnitudes of $K_{\mathrm{chem}}$ used for this set of simulations are given in the charts. Other simulation conditions were the same as in Figure 1.

In scenarios with electrochemical systems experiencing a substantial electron transfer rate, the *surface electrochemical mechanism associated with an irreversible chemical reaction of initial reactant Ox (mechanism 5)* presents a range of unique patterns in the calculated voltammograms that emerge at distinct chemical reaction rates. Every voltammetric curve in Figure 5 has been calculated for a fast electron exchange rate between the redox analytes and the working electrode ($K_{\mathrm{ET}} = 10$). In general, an increase in the chemical step rate within the range of $0.5 < K_{\mathrm{chem}} < 5$ results in a significant diminishment in the potential separation between forward and backward SWV peaks. Intriguingly, within this chemical rate range ($0.5 < K_{\mathrm{chem}} < 5$), there's an observed increase (rather than the anticipated decrease) in both the reduction and reoxidation current components as $K_{\mathrm{chem}}$ 's magnitude increases. For example, the reduction component's peak current rises severalfold, while the reoxidation component also sees a notable surge when $K_{\mathrm{chem}}$ increases from 0.1 to 5.0 (compare Fig. 5b with Fig. 5e, for example). Analogous phenomena have been witnessed in surface electrode mechanisms linked with subsequent chemical reactions, with comprehensive explanations for these phenomena found in this work and elsewhere.[1,11] As reported previously, SW voltammograms of redox systems featuring large rates of electron transfer display minor detected currents due to the swift conversion of the majority of Ox(ads) species into Red(ads) within the "dead-time" of the applied potential pulses.[11] Given that SWV measures the current at the end of the SW pulses, only a minimal initial redox form of Ox(ads) remains available for electrochemical transformation at the SW pulse's conclusion (as seen in curve 1 of Fig. 5a). However, if the molecules of initial redox form Ox(ads) participate in a concurrent chemical reaction, the chemical reaction's rate can offset the electrochemical equilibrium established during the potential pulses' "dead-time", allowing a new equilibrium to be re-established. Consequently, more Ox(ads) species might persist, available for redox transformation at the electrode surface during the SWV's current sampling pulses.[11] As a result, all SWV current components increase in the region of moderate chemical step rates, as displayed in the voltammograms in Figures 5b–e. Ultimately, for chemical step rates described by $K_{\mathrm{chem}} > 5$ (as seen in Fig. 5f), there's a resulting decrease in both the reduction and reoxidation current components due to the rapid rise of the rate of irreversible chemical reaction. In this chemical reaction rate, zone ($0.5 < K_{\mathrm{chem}} < 5$), voltammetric curve behavior mirrors that of the surface CE mechanism (refer to Figs. 4b–d). Similar to the surface CE mechanism (4), such a sequence of events leads to the vanishing of the split in net SW voltammograms of mechanism (5). However, a defining characteristic of the electrochemical mechanism involving the irreversible chemical deactivation of the initial electrochemically active reactant (mechanism 5) is the absence of the reappearance of the splitting phenomenon at higher $K_{\mathrm{chem}}$ values, unlike the surface CE mechanism (refer to Fig. 4f, for example). Indeed, the features in Figure 5's voltammetric curves can serve as valuable indicators to identify mechanism (5), characterized by high electron transfer rates, and to clearly distinguish it from mechanisms (4) and (2) considered in this work.

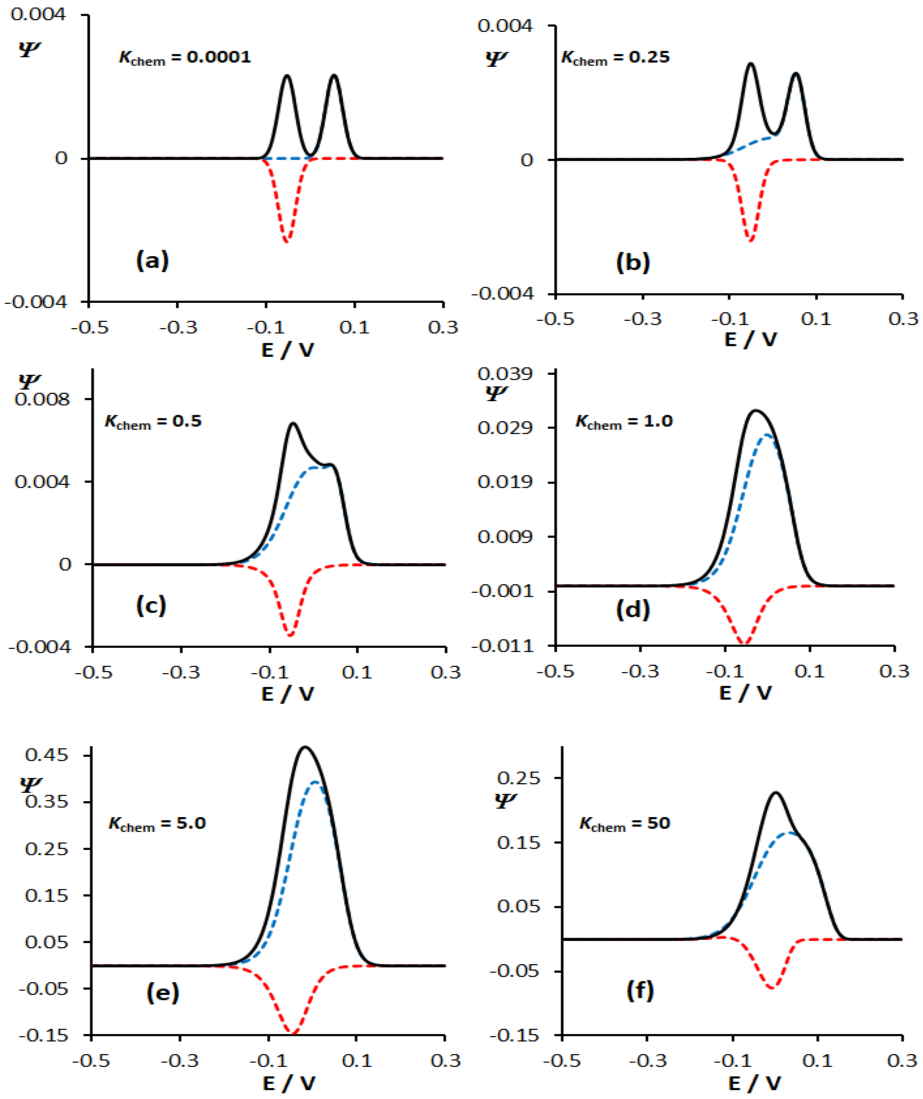

**Fig. 5**. Square-wave voltammograms of surface electrochemical mechanism (5) associated with an irreversible chemical step to give the initial electroactive form Ox, calculated as a function of the dimensionless chemical rate parameter $K_{\mathrm{chem}}$. The value of the dimensionless rate parameter of electron transfer was set to $K_{\mathrm{ET}} = 10$. The magnitudes of $K_{\mathrm{chem}}$ used for this set of simulations are given in the charts. Other simulation conditions were the same as in Figure 1.

## 4. CONCLUSIONS

Electrochemical mechanisms associated with preceding, subsequent, regenerative, or inactivating chemical reactions frequently emerge when examining various lipophilic redox systems under voltammetric conditions. Determining the specific nature of the electrochemical mechanism is essential for applying an appropriate protocol to evaluate kinetics and thermodynamics in voltammetry. Hence, it's pivotal to devise straightforward diagnostic criteria to identify each unique surface mechanism linked to a specific chemical reaction. The "split of net peak" phenomenon, a hallmark of all surface electrochemical mechanisms characterized by a rapid electron transfer rate between the working electrode and the redox system molecules when studied in square-wave voltammetry, is employed in this research to discern particular mechanisms associated with certain chemical reactions. By assessing the attributes of the "split of net SWV peak" in relation to the chemical reaction rate, it becomes feasible to distinguish among surface mechanisms associated with follow-up, preceding, regenerative, and inactivating chemical reactions. This differentiation is achievable because the chemical rate parameter showcases different impacts on the split net SWV peaks based on the ongoing electrochemical mechanism. The patterns in Figures 1–5 can guide experimentalists in identifying specific electrochemical mechanisms associated with a given chemical reaction. It's important to highlight that the effects displayed in Figures 2 to 5 can be experimentally achieved by merely adjusting the substrate "Y" concentration because the chemical kinetics of all elaborated mechanisms are considered to be of pseudo-first order. While the chemical reaction rate is also influenced by the SW frequency, experimentalists are recommended to alter the substrate "Y" concentration to obtain the effects illustrated in Figures 2 to 5. This is advised as SW frequency analysis could yield misleading results in such systems because it simultaneously affects both the chemical reaction rate (via $K_{\text{chem}}$) and the electron transfer step rate (via $K_{\text{ET}}$). The insights provided in this study are particularly beneficial for experimentalists working in protein-film voltammetry[5–8,17–22] and in designing enzymatic amperometric biosensors.[22–24]

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