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# New Aspects of Protein-film Voltammetry of Redox Enzymes Coupled to Follow-up Reversible Chemical Reaction in Square-wave Voltammetry

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**Abstract:** Protein-film square-wave voltammetry of uniformly adsorbed molecules of redox lipophilic enzymes is applied to study their electrochemical properties, when a reversible follow-up chemical reaction is coupled to the electrochemically generated product of enzyme's electrode reaction. Theoretical consideration of this so-called "surface EC<sub>rev</sub> mechanism" under conditions of square-wave voltammetry has revealed several new aspects, especially by enzymatic electrode reactions featuring fast electron transfer. We show that the rate of chemical removal/resupply of electrochemically generated Red (ads) enzymatic species, shows quite specific features to all current components of calculated square-wave voltammograms and affects the electrode kinetics. The effects observed are specific for this particular redox mechanism

**Keywords:** enzymatic voltammetry • square-wave voltammetry • surface EC<sub>rev</sub> mechanism • kinetics of follow-up chemical reactions • standard rate constant of electron transfer

(surface EC<sub>rev</sub> mechanism), and they got more pronounced at high electrode kinetics of enzymatic reaction. The features of phenomena of "split net-SWV peak" and "quasireversible maximum", which are typical for surface redox reactions studied in square-wave voltammetry, are strongly affected by kinetics and thermodynamics of follow-up chemical reaction. While we present plenty of relevant voltammetric situations useful for recognizing this particular mechanism in square-wave voltammetry, we also propose a new approach to get access to kinetics and thermodynamics of follow-up chemical reaction. Most of the results in this work throw new insight into the features of protein-film systems that are coupled with chemical reactions.

## 1 Introduction

For more than 20 years, protein-film voltammetry (PFV) is recognized as a simple but powerful tool to study redox features of many important classes of enzymes [1–9]. While the experimental set-up in PFV is quite simple to be designed, the results of theoretical models developed to study these systems under conditions of pulse voltammetric techniques are not always easy for interpretation. The theoretical models in protein-film voltammetry give mainly mechanistic, thermodynamic and kinetic insights into the chemistry of considered redox enzymes [8–30]. If a given enzyme is firmly adsorbed on a working electrode surface forming a monolayer of non-interacting redox molecules, then its chemical features can be explored by developing theoretical models of so-called "surface redox reactions" [8–30]. In principle, the meaning of terms "simple surface" and "simple protein-film" voltammetry is the same. Because in surface redox reactions there is no mass transfer of electroactive species via diffusion, theoretical consideration of protein-film voltammetric systems should be relatively simple. However, when studied with pulse voltammetric techniques, these so-called "simple" protein-film reactions exhibit several complex phenomena. If square-wave voltammetry (shortly SWV) is applied as a working technique, then the redox behaviour of many lipophilic enzyme featuring fast

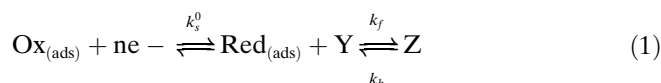
electron-exchange between working electrode and enzyme's redox active site(a) can not be considered as a simple task. The main intriguing features in such scenario are portrayed via phenomena of "split net-SWV peak" [10,14] and the "quasireversible maximum" [9,10,14–18,20–25]. We recently published a theoretical work, where we presented relevant hints about the origin of these phenomena [30]. By considering a simple surface redox reaction coupled to irreversible chemical step, it has been shown that irreversible chemical removal of electrochemically generated redox adsorbates, at the potential-pulses where they got created, might cause significant increase (and not decrease) of all current components of square-wave voltammograms. In [30], we showed that significant occurrence of both half reactions at "defined" (only "oxidation" or "only reduction") potential pulses in square-wave voltammetry is quite possible. This means: the "re-oxidation" half reaction can take place at „reduction potential pulses“, while reduction half-reaction might happen at defined „oxidation potential pulses“ in SWV. These effects might contribute significantly to the current-potential phenomena observed in simple protein-film

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square-wave voltammetric reactions [8,10,14]. In this work we present theoretical SWV results of a “simple” protein-film reaction, in which the electrochemically generated product at the working electrode surface undergoes follow-up reversible chemical transformation. The adequate description of this model is a “surface ECrev mechanism” [10,13,14]. We focus mainly on the SW voltammetric features of surface ECrev systems featuring fast rate of electron exchange between the working electrode and the redox adsorbates. Results presented in this work give new hints about the chemical behaviour of redox-active lipophilic enzymes when studied with pulse voltammetric techniques. In addition, we present new approach for getting access to kinetic and thermodynamic parameters relevant to the chemical step of surface ECrev systems.

## 2 Mathematical Model

We consider in this work theoretically the SWV features of a “simple” surface reaction ( $\text{Ox}_{(\text{ads})} + ne^- \rightleftharpoons \text{Red}_{(\text{ads})}$ ) that is coupled by a reversible follow-up chemical transformation of the electrochemically generated redox product  $\text{Red}_{(\text{ads})}$ : ( $\text{Red}_{(\text{ads})} + \text{Y} \rightleftharpoons \text{Z}$ ). The overall reaction scheme of the surface ECrev system considered is as follows:



In the mathematical model, (1) we assume that both redox forms of studied lipophilic enzyme,  $\text{Ox}_{(\text{ads})}$  and  $\text{Red}_{(\text{ads})}$ , are strongly adsorbed, while forming a monomolecular layer at the working electrode surface. We also consider that there are no interactions between the molecules of redox adsorbates, and there is no mass transfer going on by diffusion to or from the working electrode surface. By “Y” we assign a given (redox-inactive) compound present in excess in the voltammetric cell that can react only with  $\text{Red}_{(\text{ads})}$  species. In addition, we assume that the reaction between  $\text{Red}_{(\text{ads})}$  and “Y” is chemically reversible. Since “Y” is present in large excess in electrochemical cell, its concentration will be constant during electrochemical measurements. This means that the chemical step in reaction mechanism (1) is of pseudo-first order.

In the mathematical model, following initial and boundary conditions apply:

$$t = 0; \Gamma(\text{Ox}) = \Gamma^*(\text{Ox}); \Gamma(\text{Red}) = 0$$

$$t > 0; \Gamma(\text{Ox}) + \Gamma(\text{Red}) = \Gamma^*(\text{Ox})$$

$\Gamma(\text{Ox})$  and  $\Gamma(\text{Red})$  are the surface concentrations of  $\text{Ox}_{(\text{ads})}$  and  $\text{Red}_{(\text{ads})}$  species, respectively, while  $\Gamma^*(\text{Ox})$  is the total concentration of redox adsorbates at  $t=0$  at the working electrode surface. for  $t>0$ , the following conditions apply for the mechanism considered:

$$d\Gamma(\text{Ox})/dt = -I/(nFS)$$

$$d\Gamma(\text{Red})/dt = I/(nFS) + k_f\Gamma(\text{Red}) - k_b\Gamma(\text{Z})$$

By assuming the Butler-Volmer formalism at the electrode surface, we can define the current as a function of surface concentrations of redox adsorbates Ox and Red, as well as the kinetics of electron transfer rate and the applied potential in following form:

$$I/(nFS) = k_s^0 \exp(-\alpha\Phi) [\Gamma(\text{Ox}) - \exp(\Phi) \Gamma(\text{Red})]$$

Here,  $I$  is the electrical current,  $n$  is the number of electrons exchanged in an act of electrochemical transformation at the working electrode surface,  $S$  is the active surface area of working electrode, while  $\alpha$  is coefficient of the electron transfer ( $\alpha$  can have values from 0.1 to 1). The dimensionless potential  $\Phi$  is defined as  $\Phi = nF(E - E^{\circ}_{\text{Ox}(\text{ads})/\text{Red}(\text{ads})})/RT$ . In the last equation,  $F$  is the Faraday constant (96485 C/mol),  $R$  (8.314 Jmol<sup>-1</sup>K<sup>-1</sup>) is the universal gas constant,  $T$  is thermodynamic temperature, while  $E^{\circ}_{\text{Ox}(\text{ads})/\text{Red}(\text{ads})}$  is the standard redox potential of  $\text{Ox}_{(\text{ads})}/\text{Red}_{(\text{ads})}$  couple. The analytical solution of this reaction mechanism under voltammetric conditions can be found in [9–13]. The recurrent formulas for calculating dimensionless currents  $\Psi$  of theoretical square-wave voltammograms as a function of applied potential is given with:

$$\Psi_m :=$$

$$\frac{K \cdot e^{-\alpha\Phi_m} - \frac{K}{50} \cdot e^{-\alpha\Phi_m} \cdot \sum_{i=1}^{m-1} \psi_i - \frac{K \cdot K_{\text{eq}}}{1+K_{\text{eq}}} (K_{\text{chem}})^{-1} \cdot e^{\Phi_m(1-\alpha)} \cdot \sum_{i=1}^{m-1} (\psi_i \cdot M_i)}{1 + \frac{K}{50} \cdot e^{-\alpha\Phi_m} + \frac{K \cdot K_{\text{eq}}}{1+K_{\text{eq}}} (K_{\text{chem}})^{-1} \cdot e^{\Phi_m(1-\alpha)} \cdot M_1 + \frac{K_{\text{chem}}}{1+K_{\text{eq}}} \cdot e^{(1-\alpha)\Phi_m} \cdot M_1}$$

The dimensionless current  $\Psi$  is normalized as  $\Psi = I/[nFS\Gamma^*(\text{Ox})]$ . The recurrent formula is evaluated with help of numerical integration method [10]. Commercially available package MATHCAD 14 has been explored for calculating theoretical SW voltammograms.

Calculated SW voltammograms are function of the parameters of applied potential (amplitude  $E_{\text{sw}}$ , potential step  $dE$ ). In addition, theoretical SW voltammograms are affected by the electron transfer coefficient  $\alpha$ , the equilibrium constant of the follow-up chemical reaction- $K_{\text{eq}}$ , as well as by two dimensionless kinetic parameters  $K$  and  $K_{\text{chem}}$ . The dimensionless kinetic parameter  $K$  is related to the rate of heterogeneous electron transfer between the working electrode and the adsorbed redox couple. It is defined as  $K = k_s^0/f$ . Here, by  $k_s^0$  (s<sup>-1</sup>) we defined the standard rate constant of electron transfer, and  $f$  is the SW frequency. The SW frequency is defined as  $f = 1/\tau$ , where  $\tau$  is the duration of each potential SW pulse. The SW frequency  $f$  was set to 10 Hz, SW amplitude was  $E_{\text{sw}} = 50$  mV, while the potential step  $dE$  was 4 mV in all performed calculations. Additionally, calculated SW voltammograms of surface ECrev mechanism depend on dimensionless chemical parameter  $K_{\text{chem}}$ , defined as  $K_{\text{chem}} = \varepsilon/f$ , where  $\varepsilon$  is a chemical parameter (s<sup>-1</sup>) defined

as  $\varepsilon = (k_f + k_b)$ .  $k_f$  and  $k_b$  are the rate constant of the forward and backward of chemical steps of the follow-up chemical reaction. Note that dimensionless value of chemical parameter  $K_{\text{chem}}$  is related to resupply/removal of  $\text{Red}_{(\text{ads})}$  in the time-frame of potential pulses where the current is measured in SWV. In the recurrent formula,  $M$  is the numerical integration factor defined as  $M = \exp(-\varepsilon m/50) - \exp(\varepsilon(m-1)/50)$ , where “m” is the serial number of the time intervals. Equilibrium constant  $K_{\text{eq}}$  of the chemical step is defined as  $K_{\text{eq}} = k_f/k_b$ . While the value of  $K_{\text{eq}}$  defines the equilibrium amount of electroactive species  $\Gamma(\text{Red})$  at working electrode surface,  $K_{\text{chem}}$  is directly linked to the rate of chemical conversion of  $\text{Red}_{(\text{ads})}$  during the time-frame of current measurement at each potential SW pulse. In all simulations, starting potential was set to 0.25 V, and the scans were running towards final negative potential of  $-0.35$  V. We set in all calculations the value of the standard redox potential of redox couple  $\text{Ox}(\text{ads})/\text{Red}(\text{ads})$  to 0.00 V. We defined the reduction current as positive, and the oxidation current as negative. This is in line with the US electrochemical convention, as we used it in all our previous theoretical models [5,8,9,15–18]. A comprehensive overview about algorithms used can be found in [10,18].

### 3 Results and Discussion

The surface EC mechanism is considered as one of the simplest in the family of electrochemical processes coupled with chemical reactions [10]. However, in our recent work [30] we presented several sets of novel theoretical data implying huge degree of complexity even of surface EC systems that are coupled with irreversible chemical step, when these systems are studied in pulse voltammetric techniques [8–14]. The surface ECrev mechanism considered in this work is often present by many protein-film enzymatic reactions [1–7,9]. This particular mechanism appears when the electrochemically generated enzymatic form at electrode surface is unstable and reacts in a chemically reversible manner. Although several theoretical works have been published related to EC mechanism under voltammetric conditions [10–13,26,28–35], new relevant theoretical aspects of surface EC mechanism still emerge [30]. In this work, we refer to several aspects of surface ECrev mechanism featuring mainly fast electron transfer step between the working electrode and the enzymatic redox couple. If correspondingly applied, the results of this work enable the experimentalists to recognize the surface ECrev mechanism, but also to estimate relevant thermodynamic and kinetic parameters.

#### 3.1 Situation of Slow-to-moderate Rate of Electron Transfer Step of Surface ECrev Systems

Since the follow-up chemical reaction affects the surface concentration of  $\text{Red}_{(\text{ads})}$  species only, one intuitively expects that kinetics of chemical step should influence

only the re-oxidation SWV currents. However, this effect is seen to some extent at diffusional ECrev reactions. As the kinetics of follow-up chemical reaction affects mainly the “backward” SW current component (i.e. the re-oxidation current component in our case) of the diffusional ECrev systems (see voltammograms in Figure 1) [10–13], quite complex scenarios exist by SWV of “simple” surface ECrev mechanisms.

Shown in Figure 2 a–c is the effect of dimensionless chemical parameter  $K_{\text{chem}}$  to SWV current components of a surface ECrev reaction featuring moderate rate of electron transfer ( $K=0.1$ , and  $K_{\text{eq}}=1$ ).

As the rate of follow-up chemical reaction increases, we see effect of current decrease not only by re-oxidation (backward) SWV currents, but also to significant extent by the reduction (forward) SWV current components, too. Obviously, this effect needs much deeper elaboration of the particular surface ECrev reaction in SWV, as it has been recently performed with similar surface redox systems [30]. In such scenario, it is important to note that the “sigmoidal” dependencies between the net peak current and the peak-potentials of the net SWV peaks as function of equilibrium constant of the follow-up chemical step  $\log(K_{\text{eq}})$  in case of slow-to-moderate rates of electrode reaction still resemble to those reported in literature [10–13] (see Figure 3).

Significantly more complex situation exists when the electrode reaction of surface ECrev systems gets faster ( $K=0.65$ ). If  $0.50 < K < 1.05$ , for example, then we observe quite complex effect of  $K_{\text{chem}}$  to all SWV current components (see Figure 4).

In this region of  $K$  values, the rate of follow-up chemical reaction  $K_{\text{chem}}$  shows no effect to SWV current components until the values of  $K_{\text{chem}}=0.002$ . However, further increase of  $K_{\text{chem}}$ , in the region  $0.002 < K_{\text{chem}} < 0.02$ , causes increase (and not decrease) of all SWV current components (compare voltammograms of Figure 4a with that of Figure 4b). Eventually, when  $K_{\text{chem}} > 0.02$  (significant rates of follow-up chemical step), we observe “expected” features of rate of chemical step to the re-oxidation SWV current component (Figure 4c). Alongside to the decrease of re-oxidation SWV current component, in the region  $0.02 < K_{\text{chem}} < 0.1$ , considerable decrease of the reduction (forward) SWV current component is observed, too (compare voltammogram 4c with 4b). For values of  $K_{\text{chem}} > 0.1$ , the backward (re-oxidation) SWV current component gets completely diminished, and there is no effect of the chemical rate to the features of SW voltammograms any longer. Shown in Figure 5 are the dependencies of net SWV responses  $E_{\text{net,p}}$  vs  $\log(K_{\text{chem}})$  and  $\Psi_{\text{net,p}}$  vs  $\log(K_{\text{chem}})$ , simulated for several values of the dimensionless electrode kinetic parameter  $K$  and  $K_{\text{eq}}=1$ . In this scenario, the dependence  $E_{\text{net,p}}$  vs  $\log(K_{\text{chem}})$  (Figure 5a) is still in accordance to the previous findings of this mechanism [10–13]. However, for electrode reactions featuring moderate-to-fast electron transfer rates ( $0.40 < K < 1.05$ ), we observe well-defined local maxima appearing at top of sigmoidal-like curves of  $\Psi_{\text{net,p}}$  vs  $\log(K_{\text{chem}})$

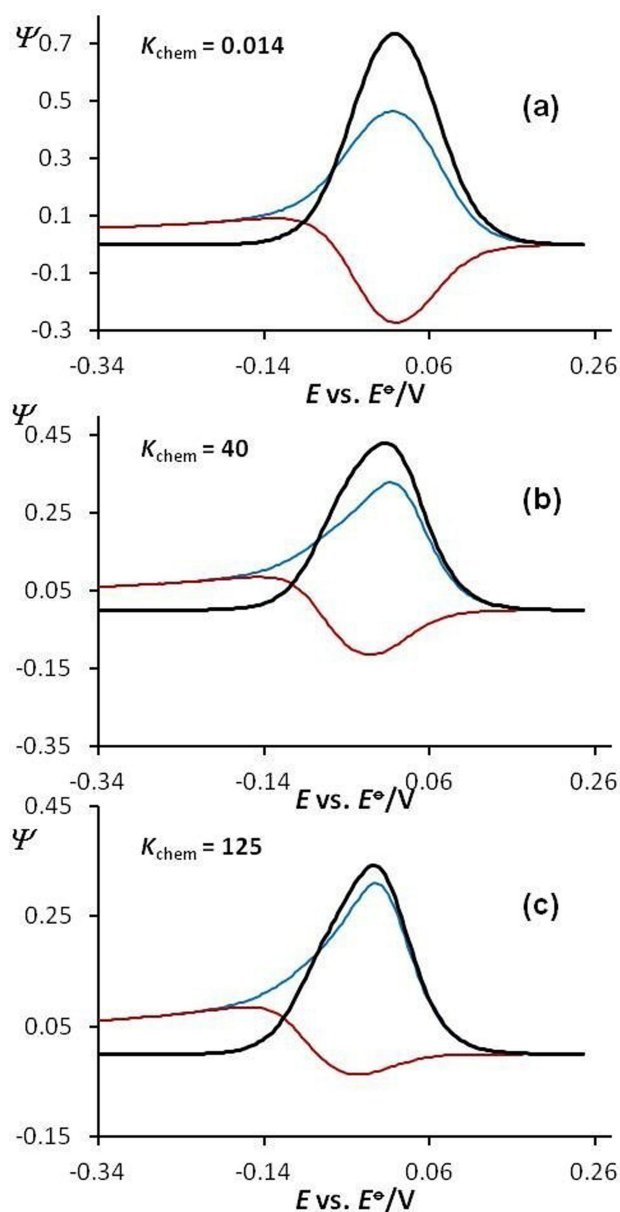


Fig. 1. Diffusional ECrev mechanism: Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) current components as a function of dimensionless chemical parameter of follow-up chemical reaction  $K_{\text{chem}}$ . Other simulation parameters were: diffusion coefficient  $D = 5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ ; dimensionless kinetic parameter of electrode reaction  $K = 0.1$ ; equilibrium constant of follow-up chemical step  $K_{\text{eq}} = 10$ ; electron transfer coefficient  $\alpha = 0.5$ ; SW frequency  $f = 10 \text{ Hz}$ ; potential step  $dE = 4 \text{ mV}$ ; square-wave amplitude  $E_{\text{sw}} = 50 \text{ mV}$ ; number of exchanged electrons  $n = 2$ ; temperature of  $T = 298 \text{ K}$ , standard redox potential of redox couple  $E^{\circ}_{\text{Ox/Red}} = 0.00 \text{ V}$ . In all simulations, the starting potential was set to  $+0.25 \text{ V}$  and the final potential was  $-0.35 \text{ V vs. } E^{\circ}_{\text{Ox/Red}}$ . Value of chemical parameter  $K_{\text{chem}}$  was set to 0.014 (a), 40 (b) and 125 (c).

dependencies (see the encircled region of curves 1 and 2 in Figure 5b). The elevation of net SWV responses in the region of  $0.02 < K_{\text{chem}} < 0.1$  is a consequence of the specific chrono-amperometric features of this mechanism in SWV.

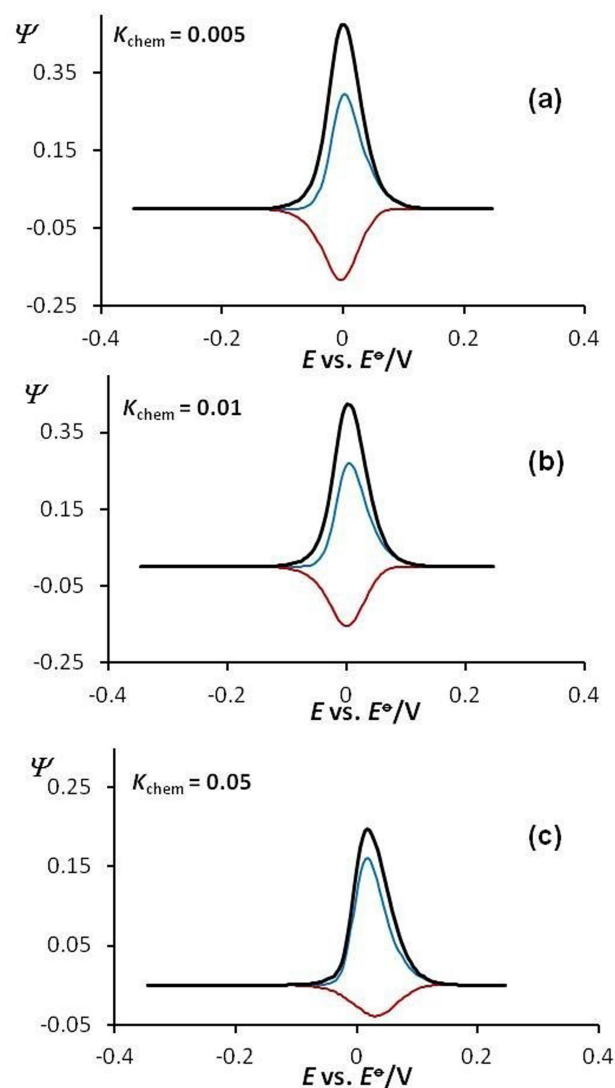


Fig. 2. Surface ECrev mechanism: Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) current components as a function of dimensionless chemical parameter of follow-up chemical reaction  $K_{\text{chem}}$ . The other simulation parameters were same as those in Figure 1. Value of chemical parameter  $K_{\text{chem}}$  was set to 0.005 (a), 0.01 (b) and 0.05 (c).

As elaborated in more details in [30], this effect appears when comparable rates exist between chemical removal/resupply of  $\text{Red}_{(\text{ads})}$  and its electrochemical generation at working electrode surface. We have shown in [30] that crucial role in such scenarios plays the rate of chemical removal of  $\text{Red}_{(\text{ads})}$ , but also of those created as a result of half-reaction  $\text{Ox}_{(\text{ads})} + ne \rightarrow \text{Red}_{(\text{ads})}$  taking place in the “dead-time” (or no-current measuring time) of defined potential SW “reduction” pulses. This effect, together with the thermodynamic possibility by surface reactions of having both half-reactions taking place simultaneously at given “only reduction” and “only oxidation” potential pulses, contributes to significant elevation of all SWV current components in region of moderate rates of

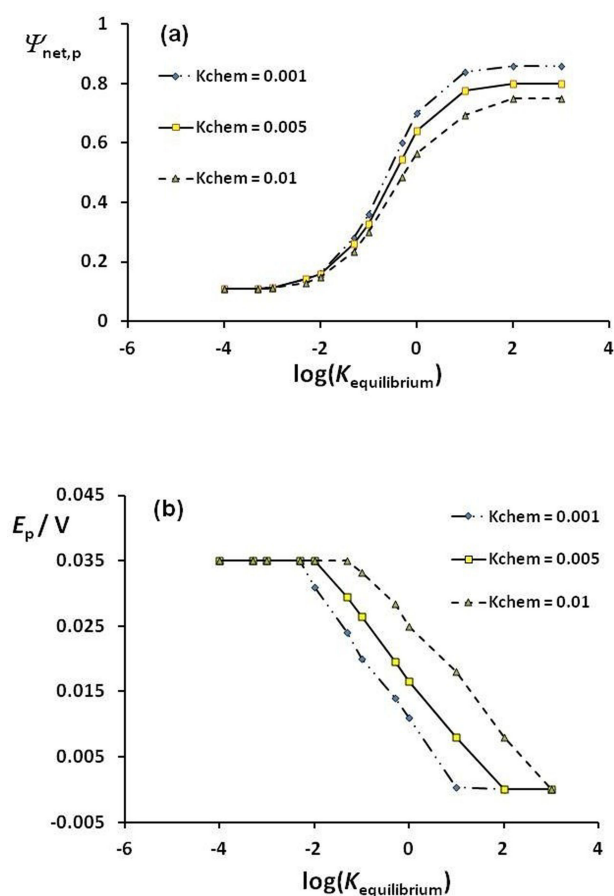


Fig. 3. Surface ECrev mechanism: (a) Dependence of the net-peak currents of simulated square-wave voltammograms (a) and of net-SWV peak potentials (b) as a function of logarithm of equilibrium constant of follow-up chemical step  $\log(K_{eq})$ . SW voltammograms were calculated for three values of chemical parameter  $K_{chem}$  of 0.001, 0.005 (b) and 0.01 (c). Other simulation parameters were same as those in Figure 2.

chemical step. The observed phenomenon in Figures 4 and 5b is a completely new aspect of surface ECrev mechanism in SWV.

Beside on the rate of electrode kinetics, this phenomenon also depends on the value of equilibrium constant of follow-up chemical step. Since in our recent work on surface ECirr mechanism we considered an irreversible chemical step, we could elaborate in [30] the effect of  $K_{chem}$  to the features of SW voltammograms only. In this work, however, situation is more complex, because we consider a reversible follow-up chemical reaction that is coupled to electrochemical step. That means, in our elaboration of the features of surface ECrev reaction in SWV, we should consider one more degree of freedom, i.e. the effect of equilibrium constant  $K_{eq}$ . Since the effects of chemical step are much more pronounced by surface ECrev systems characterized with fast electrode reaction, in our next section we focus on the effects of  $K_{eq}$  and  $K_{chem}$  to the SW voltammograms featuring fast electron transfer step.

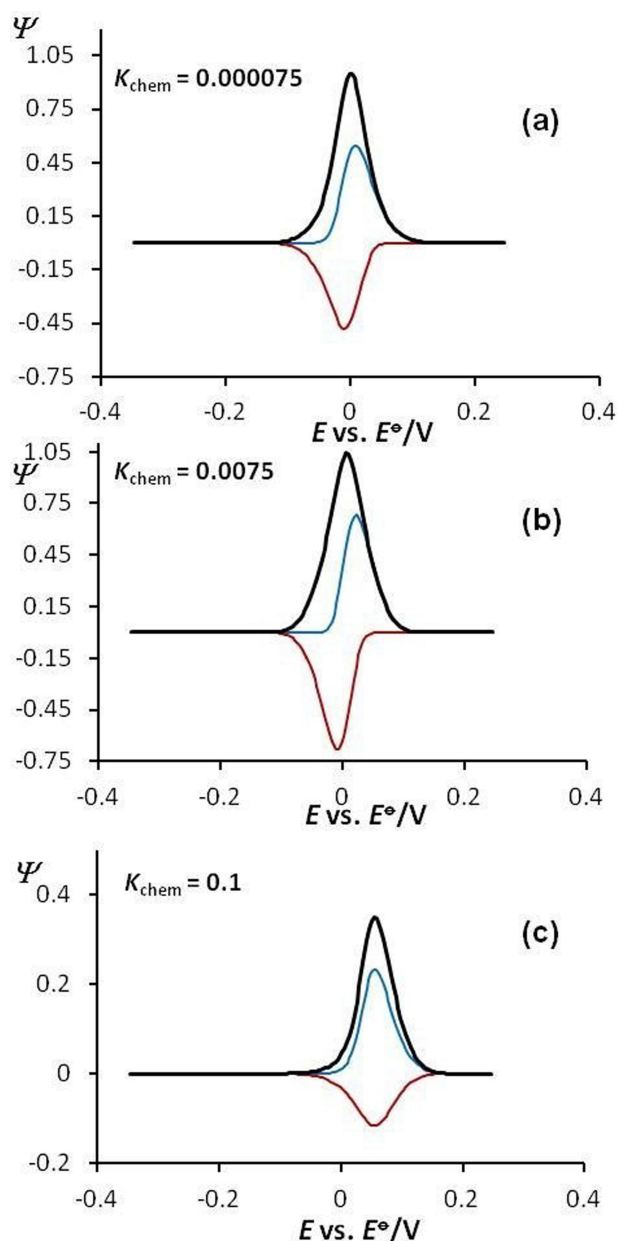


Fig. 4. Surface ECrev mechanism: Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) current components as a function of dimensionless chemical parameter of follow-up chemical reaction  $K_{chem}$ . SW voltammograms are simulated for value of dimensionless electrode kinetic parameter  $K=0.65$  (moderate rate of electron transfer). Value of chemical parameter  $K_{chem}$  was set to 0.000075 (a), 0.0075 (b) and 0.1 (c). The other simulation parameters were same as those in Figure 1.

### 3.2 Situation of fast Rate of Electron Transfer Step of Surface ECrev Systems

We start in this section considering a surface ECrev mechanism attributed with fast electron transfer step of electrode reaction, and with very slow rate of the follow-up chemical step. Shown in Figure 6 are representative

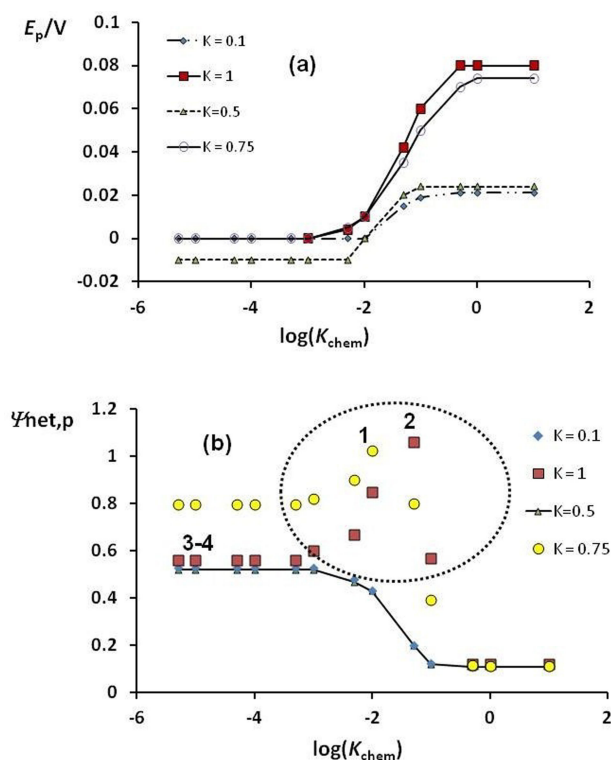


Fig. 5. Surface ECrev mechanism: (a) Dependence of the net-peak potentials of simulated square-wave voltammograms (a) and of net-SWV peak currents (b) as a function of logarithm of dimensionless chemical parameter of follow-up chemical step  $\log(K_{\text{chem}})$ . SW voltammograms were calculated for four values of dimensionless electrode kinetic parameter  $K$  of 0.1, 0.5, 0.75 and 1. Equilibrium constant of the follow-up chemical reaction was set to  $K_{\text{eq}}=10$ . Other simulation parameters were same as those in Figure 2.

SW voltammograms calculated for  $K$  of 20 (fast electrode reaction),  $K_{\text{chem}}$  of 0.000001 (very sluggish kinetics of chemical follow-up reaction) and several values of equilibrium constant of the follow-up chemical step  $K_{\text{eq}}$ . Before starting to elaborate voltammetric features portrayed in Figure 6, we should point out that the value of  $K_{\text{eq}}$  reflects the equilibrium amount of electroactive  $\text{Red}_{(\text{ads})}$  species ( $K_{\text{eq}}=k_f/k_b$ ). If value of  $K_{\text{eq}}$  is high ( $K_{\text{eq}}=200$ ), then we observe a non-perturbed SW voltammograms as those of a “simple surface” redox reaction [8,10,14] (Figure 6a). Noticeable characteristic of simple surface redox processes featuring very fast electrode transfer kinetics is the enormous current decrease and “splitting of net SWV peak” [10,14]. These phenomena happen when the rate of electron transfer is fast, so the time needed to convert  $\text{Ox}(\text{ads})$  to  $\text{Red}(\text{ads})$  at defined SW potential pulses gets shorter. If the time-period for current measurement at those potential pulses is longer than the time needed for redox transformation of  $\text{Ox}(\text{ads})$  to  $\text{Red}(\text{ads})$ , then the magnitude of the measured currents will be very small. This is a consequence of a minute amount of  $\text{Ox}(\text{ads})$  that remains “available” to be reduced to  $\text{Red}(\text{ads})$  at the end of SW potential pulses, i.e. in the tinny time-sector of

current measurements in SWV. In the same time, as the kinetics of electrode reaction increases, there is a shift of the reduction peak towards more positive potentials and of the oxidation peak to more negative potentials. This, in turn, will cause split of the net SWV response to two symmetric peaks (Figure 6a). As it is evident in Figures 6a and 6b, in some regions of the SW voltammograms, the forward and net currents are overlapping. This is because of the measuring manner in SWV and the “zero current contribution” of the backward currents in those segments [10,14]. So, in those segments of calculated voltammograms the forward current is actually the net current in the same time.

The net SW splitting phenomenon depends not only on the rate of electrode reaction, but also on the exciting signal parameters (amplitude  $E_{\text{sw}}$ , potential step  $dE$ ) [10,14]. In [14], the authors gave a list of critical values of  $E_{\text{sw}}$ ,  $dE$  by which splitting of net SW peak can be achieved for different kinetics of electrode reactions. Moreover, in [10,14], authors also provide optimal values of  $E_{\text{sw}}$ ,  $dE$  that can be used for analytical applications. Commonly, square-wave amplitude  $E_{\text{sw}}$  of 30 to 40 mV and potential step  $dE$  of 2 to 5 mV are seen as optimal parameters for analytical applications [10]. When the equilibrium constant  $K_{\text{eq}}$  gets values lower than 1, then we initially observe two remarkable features of the calculated SW voltammograms (Figure 6b and 6c): first, the current of all SW components increases by decreasing of  $K_{\text{eq}}$ ; and second, the distance between the split SW peaks diminishes by decreasing  $K_{\text{eq}}$ . The last effect is mainly caused by shifting of the re-oxidation peak towards more positive values by decreasing  $K_{\text{eq}}$ . Eventually, a further decrease of  $K_{\text{eq}}$  to value of 0.0002 leads to appearance of a single net SW voltammogram featuring a maximal SW net-peak current, and with peak-potential of the net SWV response shifted for about 100 mV more positive than the standard redox potential  $E^{\circ}_{\text{Ox}(\text{ads})/\text{Red}(\text{ads})}$  (Figure 6d). The further decrease of  $K_{\text{eq}}$  to values lower than 0.000005 produces SW voltammograms featuring significantly diminished re-oxidation current component (Figure 6e and 6f). Calculated voltammograms on Figure 6 imply that kinetics of electrode reaction of surface ECrev systems in SWV is affected significantly by available surface concentration of electroactive  $\text{Red}_{(\text{ads})}$  species. Bearing in mind this fact, it is worth to elaborate several scenarios, where we will investigate the influence of rates of follow-up chemical reaction ( $K_{\text{chem}}$ ) to the features of SW voltammograms, calculated at several different values of equilibrium constant of chemical reaction  $K_{\text{eq}}$ .

A) For fast electrode reactions ( $K=20$ ), and for large values of equilibrium constant of follow-up chemical reaction ( $K_{\text{eq}}>1$ ) of surface ECrev systems, and for values of chemical parameter  $0.0001 < K_{\text{chem}} < 0.075$  (Figure 7b and 7c) we observe an increase of both reduction and oxidation SWV current components. More pronounced is the increase of the oxidation branch of the SW voltammograms that shifts towards more positive values by increasing the value of  $K_{\text{chem}}$ .

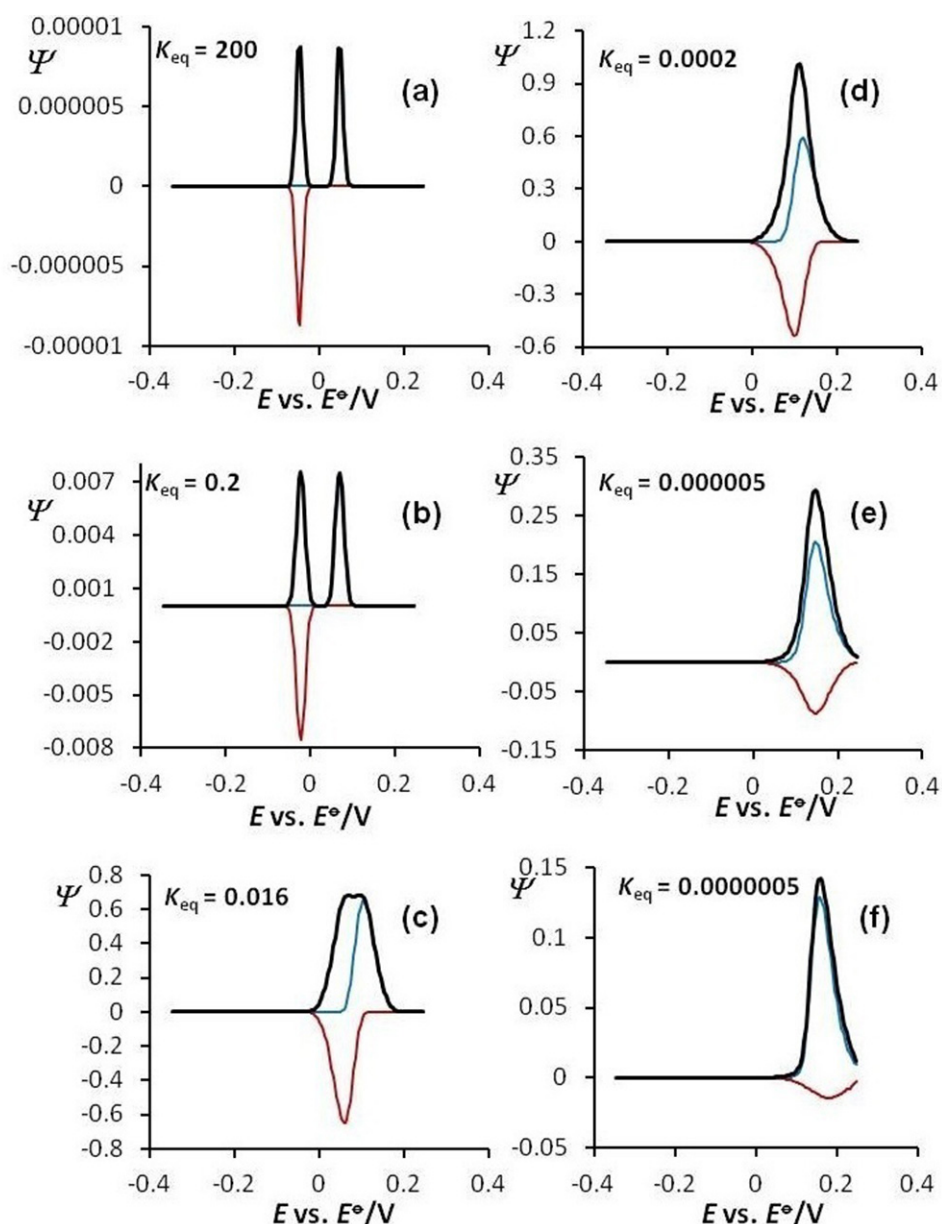


Fig. 6. Surface ECrev mechanism with fast electrode reaction: Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) currents as a function of equilibrium constant of follow-up chemical reaction  $K_{eq}$ .  $K_{eq} = 200$  (a); 0.2 (b); 0.016 (c); 0.0002 (d); 0.000005 (e) and 0.0000005 (f). Value of dimensionless chemical parameter  $K_{chem}$  was set to 0.000001, while the value of the dimensionless electrode kinetic parameter was set to  $K=20$ . The other simulation parameters were same as those in Figure 1.

The phenomenon of splitting of SWV net-peak disappears if  $K_{chem}$  gets values larger than 0.04 (Figure 7c). If dimensionless chemical parameter  $K_{chem}$  gets values bigger than 0.4, then the re-oxidation SWV current component diminishes, while there is no effect to the reduction SWV current branch (Figure 7d).

B) For fast electrode reactions ( $K=20$ ), and for value of equilibrium constant of follow-up chemical reaction of  $K_{eq}=0.01$  of surface ECrev systems, we can observe effects of the chemical rate constant to calculated SW

voltammograms at larger values of  $K_{chem}$  than in case of  $K_{eq}>1$ . In such scenario, the effect of chemical reaction rate starts to be pronounced for  $K_{chem}>0.01$  (Figure 8b). Further increase of  $K_{chem}$  leads to vanishing of split-net SWV signal, and appearance of a single voltammetric net response featuring highest current for  $K_{chem}$  of 0.05 (Figure 8c). For values of  $K_{chem}>1$ , there is no further effect of  $K_{chem}$  to the SW voltammograms (Figure 8d).

C) For fast electrode reactions ( $K=20$ ), featuring small values of equilibrium constant of follow-up chemical

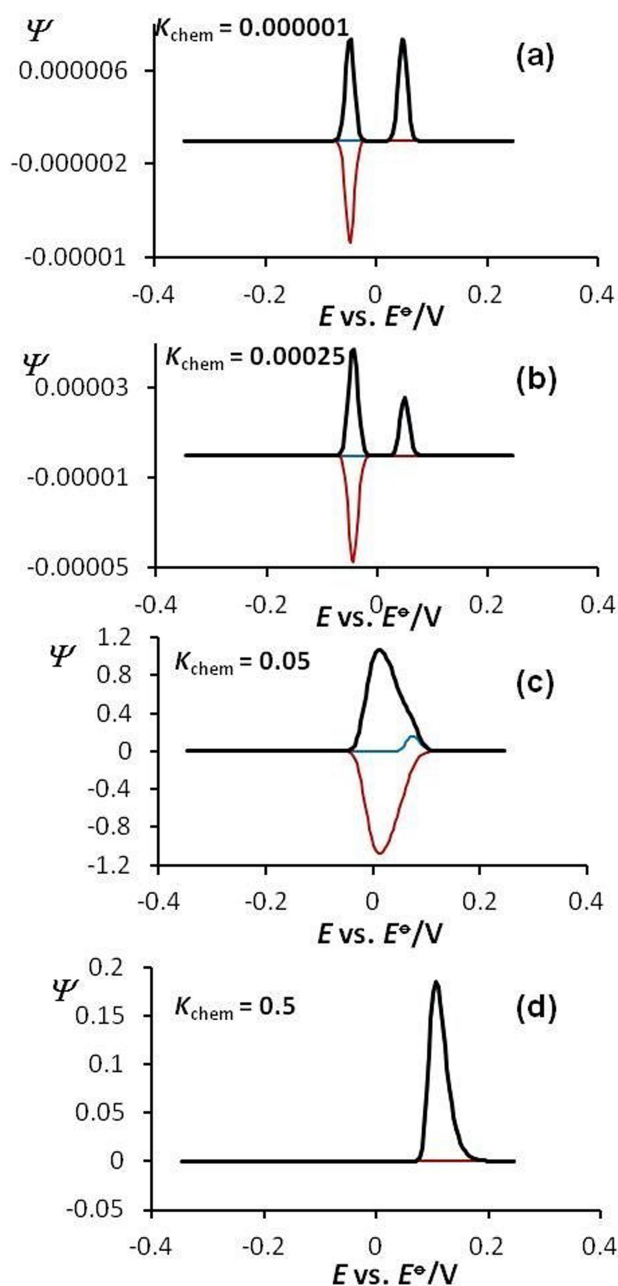


Fig. 7. Surface ECrev mechanism with fast electrode reaction and equilibrium constant of follow-up chemical reaction of  $K_{eq}=200$ : Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) currents as a function of dimensionless chemical parameter  $K_{chem}$ .  $K_{chem} = 0.000001$  (a);  $0.00025$  (b);  $0.05$  (c); and  $0.5$  (d). Value of dimensionless electrode kinetic parameter  $K$  was set to 20. Other simulation parameters were same as those in Figure 1.

reaction ( $K_{eq} < 0.0002$ ) of surface ECrev systems, there is no phenomenon of splitting of net SW voltammetric response (Figure 9a). In such scenario, we see a single SW net voltammogram, whose features are affected for significant rates of the chemical step, i.e. for  $K_{chem}$  values larger than 0.05 (Figures 9b–d).

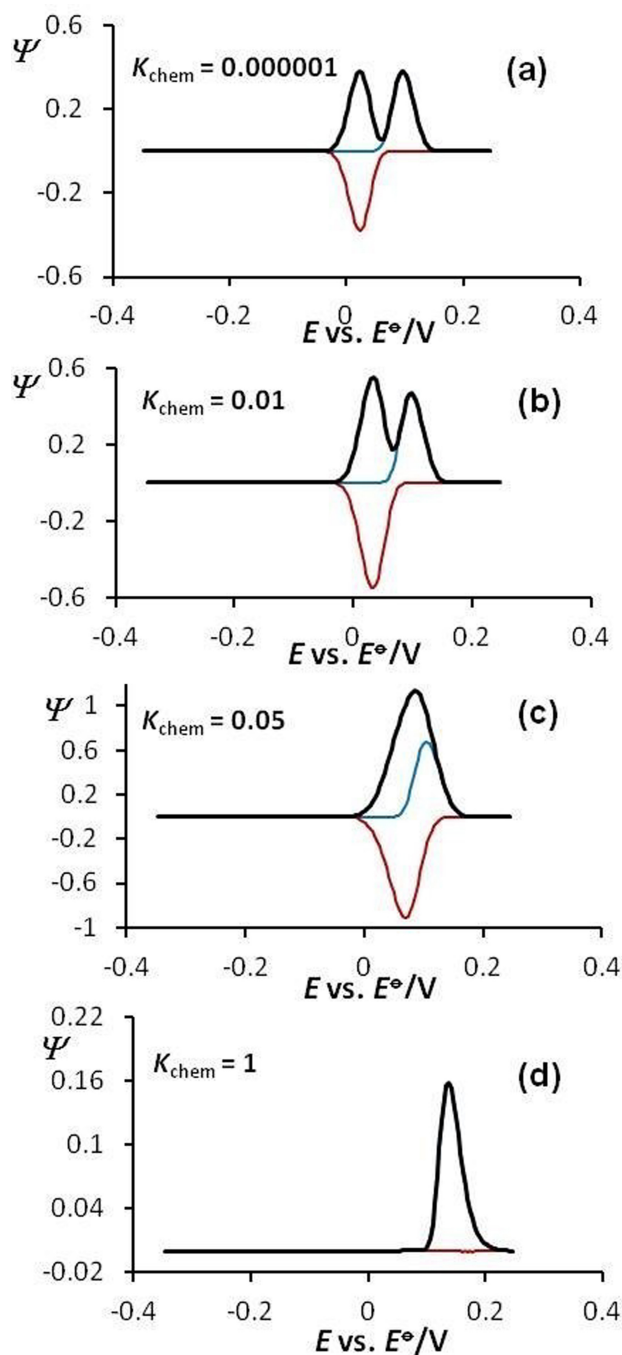


Fig. 8. Surface ECrev mechanism with fast electrode reaction and equilibrium constant of follow-up chemical reaction of  $K_{eq}=0.01$ : Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) currents as a function of dimensionless chemical parameter  $K_{chem}$ .  $K_{chem} = 0.000001$  (a);  $0.01$  (b);  $0.05$  (c); and  $1$  (d). Value of dimensionless electrode kinetic parameter  $K$  was set to 20. Other simulation parameters were same as those in Figure 1.

The SW voltammetric features described in Figures 4–9 are quite unique and they are referred for the first time in this important redox mechanism. Many of the features reported here can be used as qualitative criteria to assess



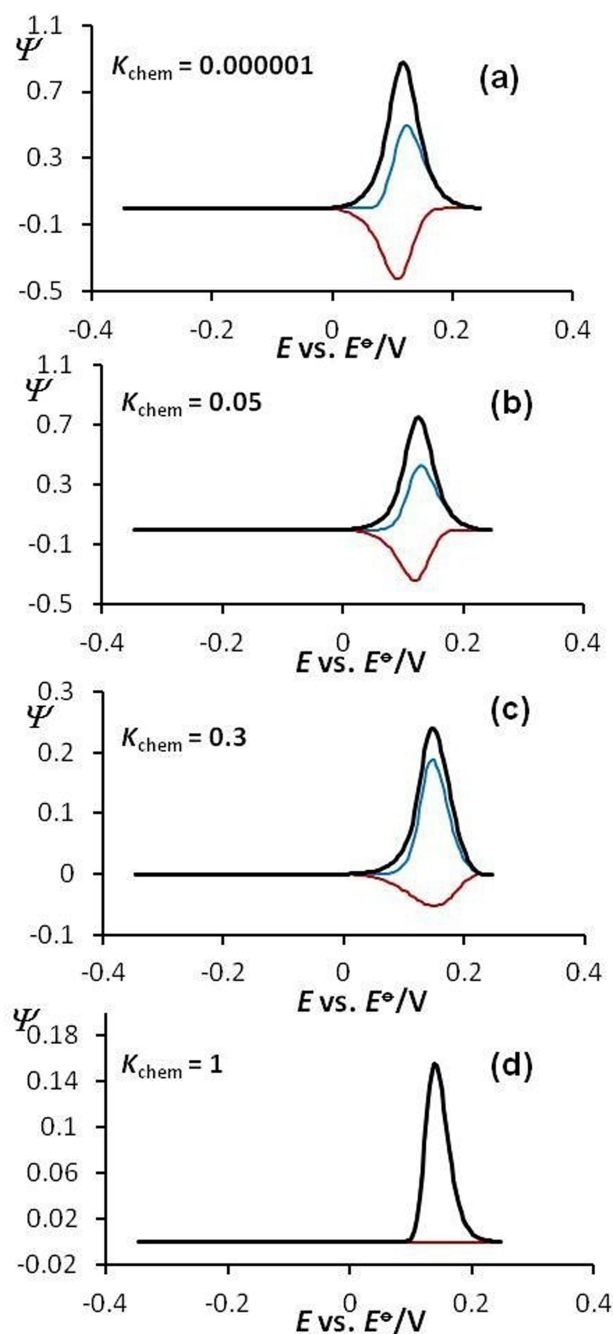


Fig. 9. Surface ECrev mechanism with fast electrode reaction and equilibrium constant of follow-up chemical reaction of  $K_{\text{eq}} = 0.0001$ : Simulated square-wave voltammograms showing the reduction (blue line), oxidation (red line) and net (black line) currents as a function of dimensionless chemical parameter  $K_{\text{chem}}$ .  $K_{\text{chem}} = 0.000001$  (a); 0.05 (b); 0.3 (c); and 1 (d). Value of dimensionless electrode kinetic parameter  $K$  was set to 20. Other simulation parameters were same as those in Figure 1.

roughly the values of  $K_{\text{chem}}$  and  $K_{\text{eq}}$ , but also distinguish the surface ECrev mechanism from similar ones, when square-wave voltammetry is used as a working technique.

## 4 Conclusions

In this work we have elaborated several new aspects of surface redox reactions, whose electrochemically generated product is involved in a follow-up reversible chemical transformation, under conditions of square-wave voltammetry. We have shown that both, the kinetics and thermodynamics of follow-up chemical reaction can cause significant changes to the SW voltammetric responses of surface ECrev systems, especially by those featuring fast electrode reactions. Since the “splitting” of net SW voltammetric response is most recognizable feature of fast surface electrode reactions, it is useful to explore this phenomenon for qualitative recognition of surface ECrev systems. As we demonstrated in Figures 3 to 9, for fast surface electrode reactions, the rate of follow-up chemical step can cause (in moderate regions of  $K_{\text{chem}}$ ) significant increase of both reduction and oxidation peak currents. This happens if equilibrium constant of chemical reaction have values larger than 0.01. Shown in Figure 10 is the dependence of the ratio of |absolute values| of peak currents of the oxidation peaks vs. peak currents of reduction peaks, calculated for  $K=2$  (situation of split net-SWV peak), and for several equilibrium constants of follow-up chemical reaction. Noticeably, for  $K_{\text{eq}} > 0.2$ , the ratio  $|\Psi_p(\text{oxidation})/\Psi_p(\text{reduction})|$  features well pronounced local maxima as a function of chemical rate constant  $\log(K_{\text{chem}})$ . These maxima exist in the region of  $0.005 < K_{\text{chem}} < 0.1$ . Since the value of  $K_{\text{chem}}$  can be altered by changing the concentration of “Y” compound in electrochemical cell, for fast surface electrode reactions, by plotting the ratio of the values of oxidation/reduction of SW peak currents as function of  $c(\text{Y})$ , we can reproduce the curves portrayed in Figure 10. Significantly elevated “sigmoidal” curves of that dependence can serve

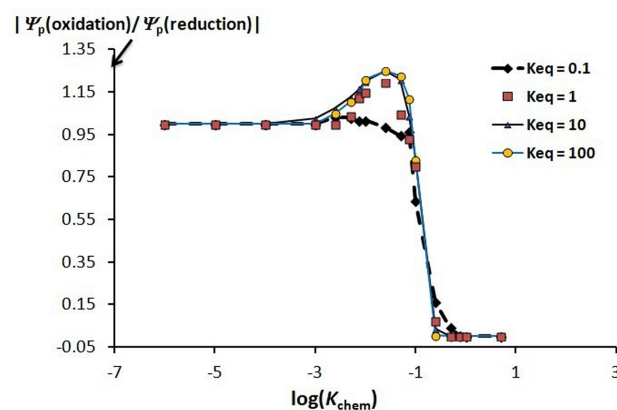


Fig. 10. Surface ECrev mechanism with fast electrode reaction: Ratio of oxidation vs. reduction peak currents of simulated square-wave voltammograms as a function of logarithm of dimensionless chemical parameter  $\log(K_{\text{chem}})$ . SW voltammograms were calculated for four values of equilibrium constant of follow-up chemical step  $K_{\text{eq}}$  of 0.1; 1; 10 and 100. Value of dimensionless electrode kinetic parameter was set to  $K=2$ . Other simulation parameters were same as those in Figure 2.

as qualitative criterion to recognize the surface ECrev mechanism. In order to estimate the value of chemical parameter  $K_{\text{chem}}$ , we can explore another specific feature of surface electrode reactions, i.e. the “quasireversible maximum” [10]. Because of the specific current-measuring procedure of square-wave voltammetry, and the specific behaviour of surface redox reactions, surface electrode processes with moderate electron transfer are featuring highest measured current [10]. The parabolic dependence between the measured net-SWV peak currents as a function of the dimensionless kinetic parameter related to the electrode reaction ( $K$ ), allows determination of standard rate constant of electron transfer step  $k_s^\circ$  in a very elegant manner [10].

Shown in Figure 11a are the “quasireversible maxima” of surface ECrev mechanism, constructed for  $K_{\text{eq}}$  of 1, and for several values of dimensional chemical rate parameter  $K_{\text{chem}}$ . Obviously, an increase of  $K_{\text{chem}}$  from 0.01 to 0.09 is followed by shifting of the position of the “quasireversible maxima” towards higher values of  $K$ . Recall that the dimensionless electrode kinetic parameter  $K$  is defined as  $K = k_s^\circ / f$ , where  $f$  is the SW frequency. In addition, the

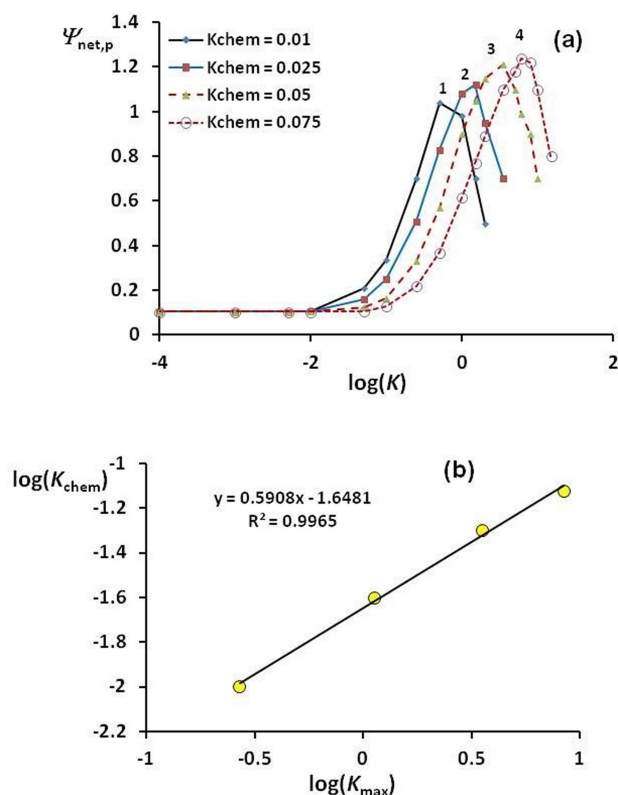


Fig. 11. Surface ECrev mechanism with slow and moderate electron reactions and equilibrium constant of follow-up chemical reaction of  $K_{\text{eq}}=1$ . (a) “Quasireversible maxima” simulated for four different values of dimensionless chemical parameter  $K_{\text{chem}}$ .  $K_{\text{chem}}$  was set to 0.01 (1); 0.025 (2); 0.05 (3) and 0.075 (4). (b) Dependence of  $\log(K_{\text{chem}})$  as a function of logarithm of maximal values of dimensionless electrode kinetic parameter  $\log(K_{\text{max}})$ . Other simulation parameters were same as those in Figure 2.

peak currents corresponding to the “quasireversible maxima” get also higher values in a same way by increasing  $K_{\text{chem}}$ . Moreover, there is a linear dependence between  $\log(K_{\text{chem}})$  and  $\log(K_{\text{max}})$ , characterized with correlation coefficient of  $R^2$  bigger than 0.99.

The slope of dependence  $\log(K_{\text{chem}})$  vs.  $\log(K_{\text{max}})$  in Figure 11b allows determination of the rate of chemical step  $K_{\text{chem}}$ . Experimentally, the “quasireversible maxima” at Figure 11a can be reproduced by altering the concentration of electro-inactive “Y” reactant in the electrochemical cell, and by plotting the dependence of measured SWV net-peak currents as a function of  $\log(f^{-1})$ , for defined concentration of  $c(\text{Y})$  in electrochemical cell. In such scenario, the position of quasireversible maxima should shift toward higher values of  $\log(f^{-1})$  as  $c(\text{Y})$  increases. By using the corresponding equation of the linear dependence in Figure 11b, we can estimate the value of the chemical parameter  $K_{\text{chem}}$ . If we plot the peak currents of the forward (reduction) SWV peaks vs.  $\log(K)$ , measured for several concentrations of chemical agent  $c(\text{Y})$ , then we can also extract the value of forward rate constant  $k_f$  in a similar manner as that shown in Figure 11. Consequently, from the estimated values of  $K_{\text{chem}}$  and  $k_f$ , and knowing the fact that  $K_{\text{chem}} = (k_f + k_b)/f$ , we can also determine value of  $k_b$ . If values of  $k_f$  and  $k_b$  are once estimated, we can also calculate the value of equilibrium constant  $K_{\text{eq}}$ , since  $K_{\text{eq}} = k_f/k_b$ . To achieve this, we also need to know the value of  $k_s^\circ$ . For estimating standard rate constant of electron transfer of the electrode reaction  $k_s^\circ$ , as well as the electron transfer coefficient  $\alpha$ , one can use some of the procedures elaborated in [8–10,13,14]. The methodology presented here is a completely new approach of getting access to kinetics and thermodynamics of follow-up chemical step by the surface ECrev mechanism.

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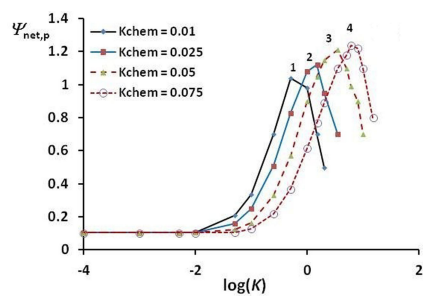
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**New Aspects of Protein-film Voltammetry of Redox Enzymes Coupled to Follow-up Reversible Chemical Reaction in Square-wave Voltammetry**

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