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Review

Analysis of Drug-Drug Interactions with Cyclic Voltammetry: An Overview of Relevant Theoretical Models and Recent Experimental Achievements

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Abstract- In this review, we focus on cyclic voltammetry as a reliable electrochemical technique to study mechanisms, kinetics and thermodynamics of various types of drug-drug interactions. While we present and discuss six theoretical models relevant to analyze drug-drug (or drug-DNA) interactions, we also give hints about recent experimental achievements in this field. In addition, we provide the readers several sets of simulated voltammograms and data in tabular form, which can be used to recognize particular mechanism of drug-drug interactions in cyclic voltammetry. Moreover, we give hints to the voltammetric procedures that allow access to kinetics and thermodynamics parameters, which are relevant to various types of drug-drug interactions. The results we present in this work can be of big help to the scientists working in the field of pharmacy, pharmacology, medicinal chemistry and bio-electrochemistry.

Keywords- Cyclic voltammetry; Thermodynamics of drug-drug interactions; Kinetics of drug-drug interactions; Stability constant of complexes; Electrode mechanisms

1. INTRODUCTION

Administration of a given drug in combination with another drug can lead to significant interactions between the active compounds. Information about strength of drug-drug interactions, and the pharmacokinetic features of defined drugs, can help to define most suitable

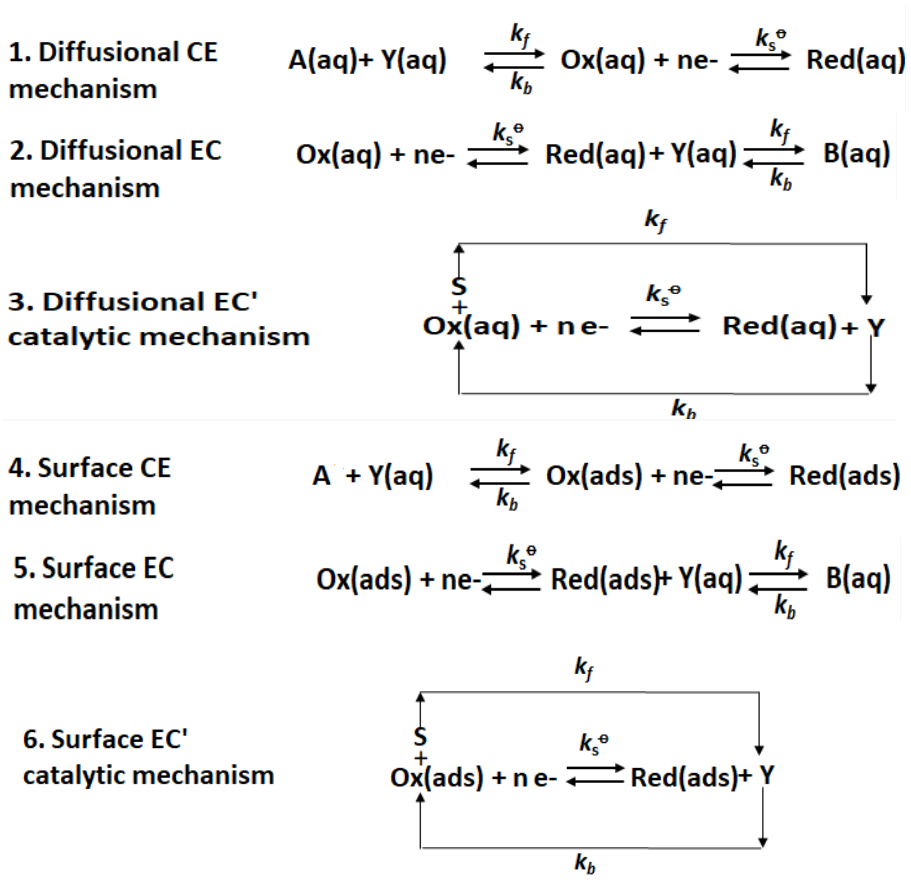
way of their administration. By analyzing the chemical interactions between two drugs, we can get information about stability, efficiency and the possible toxic effects related to the combination of drugs we administrate. When interactions between two drugs (or a given drug and DNA) are considered, one should always get information about: (a) mechanism of interactions between the drugs and the factors affecting the strength of drug-drug interactions; (b) kinetics of drug-drug interactions; (c) thermodynamics of drug-drug interactions. While the thermodynamic parameters relevant to drug-drug interactions give information about the magnitude of equilibrium (stability, or binding) constant, the kinetics of the interactions between two drugs can reveal information relevant to possible synergetic or antagonistic effects. In the last 40 years, voltammetry became a simple, fast, cheap and reliable tool for getting insight into mechanisms, kinetics and thermodynamics of interactions of many drugs [1-4]. We also witness that cyclic voltammetry (CV) is particularly suitable electrochemical techniques to analyze the drug-drug interactions from various aspects [1-3, 5]. In this short review we present: (a) theoretical results from mathematical models in CV, relevant to study mechanisms, kinetics and thermodynamics of drug-drug interactions and (b) experimental achievements performed by CV in analysis of some drug-drug or drug-DNA interactions. We consider systems in which interactions between two hydrophilic drugs occur, but also systems where one highly lipophilic and one hydrophilic drug undergo a chemical reaction. As we present several sets of voltammograms simulated for all relevant mechanisms considered, we also give hints to the theoretical methodologies proposed to analyze various drug-drug interactions. The results presented in this work are quite relevant for all experimentalists working in the field of drug-drug interactions studied with electrochemical techniques. Since there are plenty of review works dedicated to various amperometric biosensors for pharmaceutical applications, we skip in this mini-review elaborating papers concerned about construction of biosensors designed for drug quantification.

2. DISCUSSION

2.1. Theoretical models relevant to analyze drug-drug and drug-DNA interactions in cyclic voltammetry

We consider in this part several electrode mechanisms that are relevant to describe interactions between two drugs. Under the term “electroactive” or (electrochemically active) we define a drug or a drug-metabolite (the last is generated chemically or electrochemically) that can undergo electrochemical transformation at the working electrode surface. When we use the term “electrochemically inactive”, we will consider a defined participant in an electrode mechanism that does not show electrochemical activity in the range of applied potentials. The symbol “C” at all mechanisms comprises “chemical reaction”, while the symbol “E” stands for “electrochemical reaction”. The term C’ denotes so-called “catalytic (regenerative) chemical

reaction". The term "diffusional" means that the mass transfer of electroactive species occurs via diffusion only. The term "surface" suggests that the electroactive species are strongly adsorbed at the working electrode surface and there is no mass transfer taking place by diffusion. With "Ox" we assign the oxidized electroactive form of a given drug or metabolite, while "Red" stands for the reduced electroactive form. We use the symbols "A", "Y" and "S" to assign electrochemically inactive drugs (or DNA) that can enter in some type of chemical interactions with the electrochemically active forms of the drug (Ox or Red). In mechanisms 3 and 6, we assume that "S" and "Y" drugs do not react between them. In mechanisms 4, 5 and 6 we suppose that there are no chemical interactions between the adsorbed species. Terms "aq" and "ads" stand for aqueous solution (dissolved in water) and adsorbed state, respectively. At all considered mechanisms we assume that reversible chemical reactions are coupled to the "quasi-reversible" (kinetically controlled) electrochemical step. The assignation and schematic representation of the elaborated mechanisms is as follows:



In all considered mechanisms, k_f is the rate constant of forward chemical reaction, while kb is the rate constant of backward chemical step. With k_s^{Θ} we assign the standard rate constant of electron transfer related to reaction of electrode transformation (units of k_s^{Θ} are cm s^{-1} at diffusional mechanisms, and s^{-1} at surface mechanisms). It is worth to emphasize that in all elaborated mechanisms, the chemical rate constants are considered to be of pseudo first order. This means that we have one of the electrochemically inactive drugs ("A", "S" or "Y") present

in large excess in voltammetric cell. In all mechanisms, we assume constant values of parameters related to the electron transfer step (electron transfer coefficient α=0.5; diffusion coefficients of both Ox and Red is equal to D=5×10^{-6} cm^{2}s^{-1}). Dimensionless kinetic parameter related to electron transfer step K_{ET} is defined as: K_{ET}=k$_s^{\Theta}$ τ$^{0.5}$$D^{-0.5}$ at all diffusional mechanisms (1-3), and K_{ET} = k_s^{Θ}τ at all surface electrode mechanisms (4-6). All simulations in this work are performed at constant measuring time of potential steps τ=0.01s. This implies that we consider experiments in which scan rate is kept constant, and the rate of chemical reactions is modified via altering concentration of electrochemically inactive drugs "A", "Y", or "S".

At this point, it is worth to emphasize that the current we measure in voltammetry (under the applied potential) is due to reaction Ox + ne$^-$ ↔ Red (i.e. due to exchange of electrons between working electrode and the electroactive drugs species). However, the chemical changes in concentrations of Ox and Red that will occur in the time frame of current measuring windows, commonly cause specific features at the recorded cyclic voltammograms. Indeed, the features of current-potential curves of every considered mechanism will depend on the current-measuring time scale, on the nature of chemical reaction, and on the kinetics and thermodynamics parameters related to the chemical step. At the beginning of the theoretical part, we elaborate some of the most relevant theoretical features of all considered mechanisms under conditions of cyclic staircase voltammetry. In so-called "surface" electrode mechanisms 4-6, we assume that the redox active forms of the drugs Ox(ads) and Red(ads) are strongly adsorbed (ads) at the working electrode surface, and there is no mass transfer occurring via diffusion. In addition, we assume that there are no interactions between the adsorbed species.

2.1.1. Diffusional CE mechanism

This specific electrode mechanism is met when a chemical conversion of initial electrochemically inactive drug "A" creates a water-soluble electrochemically active reactant Ox. For constant scan rate, and for constant molar concentration of drug "A", the voltammetric features of this electrode mechanism depend on two dimensionless parameters related to the chemical step: (a) the value of equilibrium constant Keq = k_f/k_b; and (b) the kinetic of the chemical reaction expressed via dimensionless chemical parameter $K_{chemical}$, defined as $K_{chemical}$ = (k$_f$+k$_b$)τ. While the value of Keq defines the initial amount of Ox available for electrode transformation, the magnitude of chemical parameter $K_{chemical}$ reflects the influence of the rate of chemical reaction relative to the time scale of voltammetric measurements τ. Actually, in experiments where one is concerned to quantify the drug-drug interactions, determination of the values of Keq and $K_{chemical}$ (i.e. k_f and k_b) is a crucial segment. These parameters are directly related to the strength of interactions (via k_f and k_b), and to the magnitude of thermodynamic constant of given drug-drug chemical equilibrium (via Keq). If Keq > 100, we get unperturbed cyclic voltammograms as of a simple electrode reaction Ox(aq) + ne$^-$ ↔ Red(aq). Under such conditions, initial amount of Ox(aq) created via chemical

preceding reaction is large enough, so the kinetics of preceding chemical reaction does not play any significant role in the current-measuring time segment at potential steps. If *K*eq ≤1, then we witness obvious effects of the kinetics of preceding chemical step to the features of simulated voltammetric patterns. Shown in figure 1 and 2 is a series of cyclic voltammograms of a diffusional CE mechanism, simulated for *K*eq of 0.0001 (Figure 1) and *K*eq of 0.15 (Figure 2).

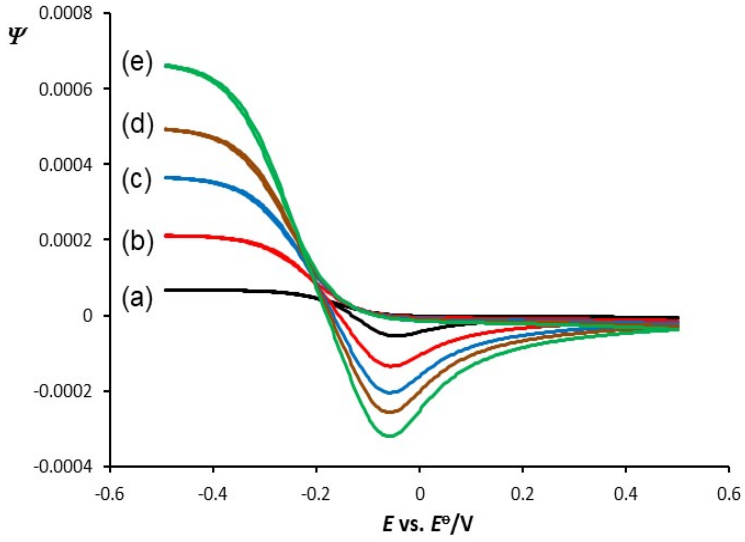


Figure 1. Influence of the chemical parameter $K_{chemical}$ to the features of cyclic voltammograms of a diffusional CE mechanism simulated for small value of equilibrium constant *K*eq = 0.0001. Value of dimensionless kinetic parameter of electron transfer was K_{ET} = 0.5, electron transfer coefficient was set to α= 0.5, height of potential step was d*E* = 4 mV; duration of potential step was τ = 0.01 s, temperature T = 298 K, while the diffusion coefficient of Ox and Red were set to equal value of D = 0.000005 cm^2s^{-1}. The value of chemical rate parameter $K_{chemical}$ were: 40 (a); 140 (b); 240 (c); 340 (d) and 440 (e).

Voltammetric patterns in figure 1 are simulated at high rates of the preceding chemical step. Under such circumstances, cyclic voltammograms feature well-developed anodic (oxidative) current component, and a steady-state (plateau-like) forward (reductive) current branch. This voltammetric feature is typical for CE systems with $K_{chemical} \times K$eq > 10, but for *K*eq < 0.01. In such scenario, the magnitude of limiting currents corresponding to the plateau-$I_{limiting}$ is proportional to $(K_{chemical})^{0.5}$, and independent on scan rate. In this kinetic region, the slope of $I_{limiting}$ vs. $(K_{chemical})^{0.5}$ equals to: $nFSc^*(\tau^{-1}DK_{eq})^{0.5}$. The dependence between $I_{limiting}$ vs. $(K_{chemical})^{0.5}$ can be explored for the determination of Keq, as described in [2, 5-7], providing that value of diffusion coefficients of Ox and Red (D) is known. The value of *K*eq can be also accessed from the slope of the dependence of mid-peak potentials $E_{mid,p}$ vs. log($K_{chemical}$), in regions of $K_{eq} < 10$, and at high values of $K_{chemical}$ as described in [2, 6, 7].

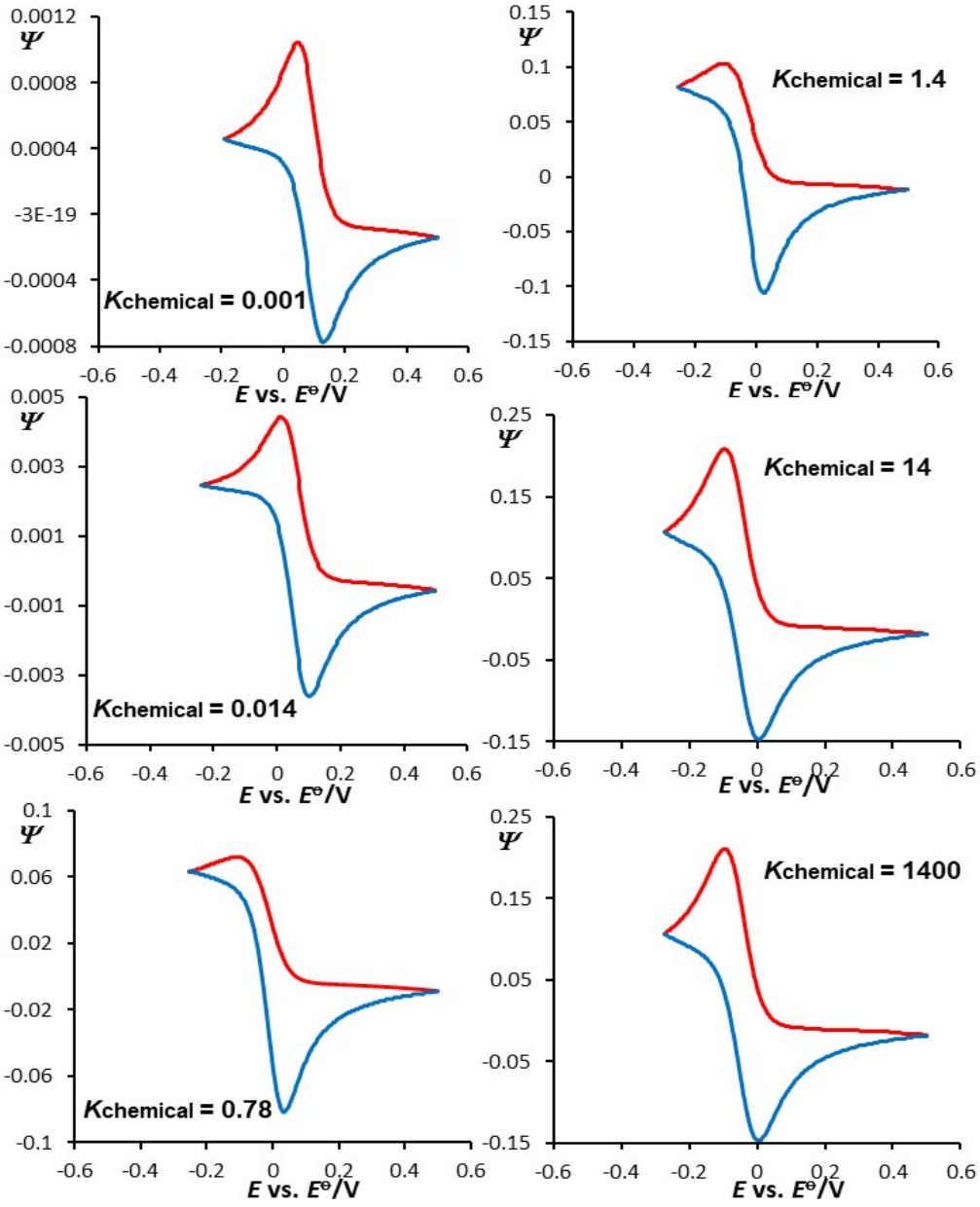


Figure 2. Influence of the chemical parameter $K_{chemical}$ to the features of cyclic voltammograms of a diffusional CE mechanism simulated for value of equilibrium constant Keq = 0.15. Other simulation parameters were same as those in figure 1. Values of the chemical rate parameter $K_{chemical}$ are given in the graphs.

If K_{eq} is small ($K_{eq} < 0.1$), and if rate of chemical step is also small ($_{Kchemical} < 0.1$), then the current is governed by the electrode reaction Ox(aq) + ne$^-$ ↔ Red(aq). Under such circumstances, the value of the mid-peak potential $E_{mid,p}$ shifts for (-59mV/n) per every tenfold increase of $_{Kchemical}$ [2, 6], with intercept of that dependence being equal to: $E^{\Theta}_{Ox/Red}$ + (0.059V/n)log[Keq/(1+K_{eq})]. The intercept of $E_{mid,p}$ vs. log($K_{chemical}$) dependence in this kinetic region can give access to the magnitude of Keq, providing that the value of $E^{\Theta}_{Ox/Red}$ is known.

In case of moderate values of *K*eq, the chemical parameter $K_{chemical}$ produces complex phenomena to voltammetric patterns, as presented in figure 2. In such scenario, there is a mixed kinetic control, and we have to make a "two-parameter fitting procedure" [2] or we have to know *K*eq from independent experiments (spectrophotometric, for example) in order to evaluate the magnitude of chemical rate parameter $K_{chemical}$ [2, 6].

2.1.2. Diffusional CE mechanism

This mechanism is met when an electrochemically generated drug-product Red(aq) enters in a follow-up chemical reaction with an electrochemically inactive drug "Y" in a reversible fashion. We designate this system as "diffusional ECrev mechanism" (or simply EC mechanism), providing that the mass transfer of Ox and Red to the working electrode takes place via diffusion. For constant values of the diffusional coefficients of Ox and Red, and at constant scan rate, the features of calculated cyclic voltammograms depend on dimensionless parameters *K*eq and $K_{chemical}$. These parameters are defined identically as for diffusional CE mechanism elaborated in previous section. If the equilibrium constant is large (i.e. *K*eq > 100), then the entire mechanism turns to ECirr, or electrochemical reaction coupled with irreversible chemical reaction. In that scenario, an increase of $K_{chemical}$ produces voltammetric patterns as those depicted in Figure 3. Any increase of $K_{chemical}$ in the region $K_{chemical} > 0.001$ (for *K*eq > 100) leads to diminishment of the current of backward (reoxidation) branch of cyclic voltammograms. The ratio between anodic $I_{p,a}$ and cathodic $I_{p,c}$ peak current features sigmoidal dependence on $\log(K_{chemical})$. Obtained sigmoidal curves of $I_{p,a}/I_{p,c}$ vs. $\log(K_{chemical})$ can be used for the determination of $K_{chemical}$. In the kinetic region, the mid-peak potential of cyclic voltammograms shifts 59 mV/n for a tenfold increase of $K_{chemical}$. For small and moderate values of *K*eq, we observe a sigmoidal dependence of $E_{mid,p}$ vs. $\log(K_{chemical})$, with linear parts of the curves having slopes of -59 mV/n, and intercepts that are function of *K*eq. The slope of $E_{mid,p}$ vs. $\log(K_{chemical})$ in kinetic region is defined as: $E^{\Theta}_{Ox/Red}+[2.3RT/(nF)]\log(1+Keq)$. The last parameter can be explored for the determination of *K*eq, if the value of $E^{\Theta}_{Ox/Red}$ is known [2]. In the case when *K*eq < 1, and $K_{chemical} > 0.1$, one observes complex kinetically controlled voltammetric patterns. Under such conditions, only by "two-parameters fitting" procedure [2] one can get access to $K_{chemical}$ and *K*eq.

2.1.3. Diffusional EC' mechanism

The catalytic EC' mechanism coupled with an irreversible chemical reaction is considered as a very suitable model to study interactions between many drugs and other physiologically important substances [2, 5, 7-23]. We elaborate here a more comprehensive type of Electrochemical-Catalytic i.e., the ECrev' mechanism, where we suppose a reversible chemical

step coupled to electrode reaction. The mechanism we consider provides access not only to the kinetics, but also to thermodynamics of drug-drug interactions, as reported in [24, 25].

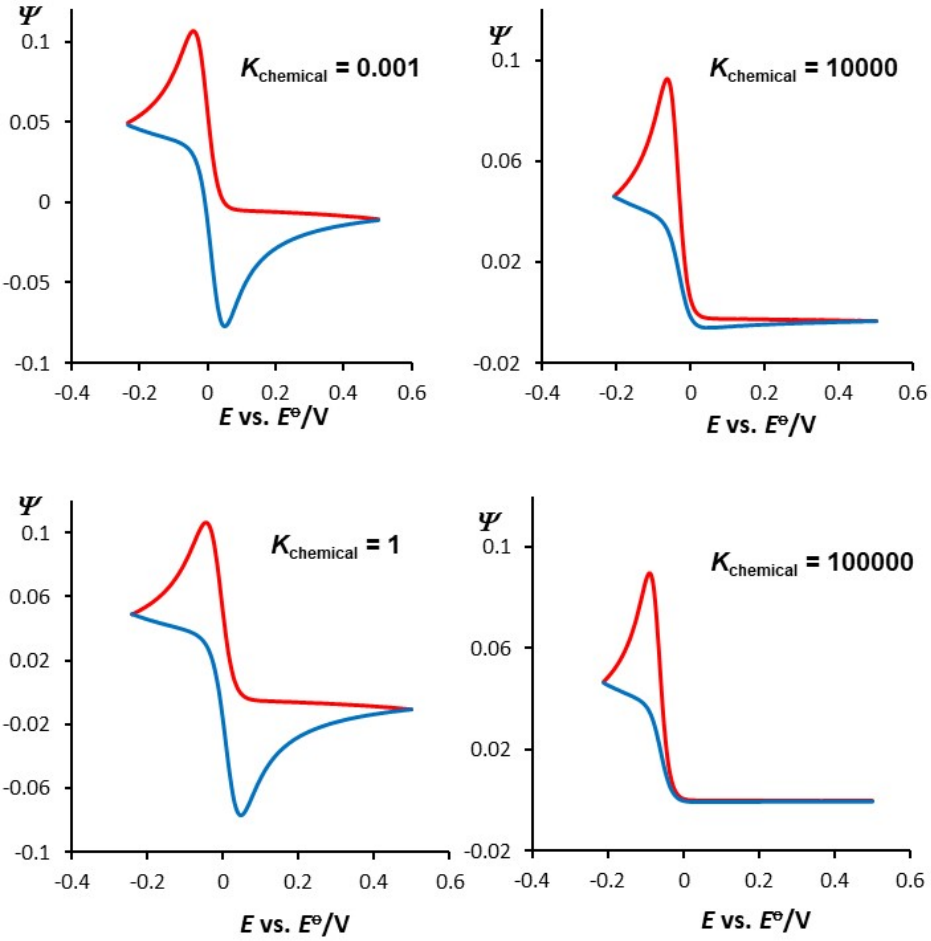


Figure 3. Influence of the chemical parameter $K_{chemical}$ to the features of cyclic voltammograms of a diffusional EC mechanism simulated for value of equilibrium constant Keq = 1000. Other simulation parameters were same as those in Figure 1. Values of the chemical rate parameter $K_{chemical}$ are given in the graphs.

In this mechanism, we assume both "electroactive" compounds (the two redox forms of same drug) to enter into a reversible chemical interconversion from the beginning of experiment, even before potential is applied. We assume that Ox(aq) undergoes selective chemical reaction with the substrate (drug) "S", and creates compounds Red(aq) and "Y" in a chemically reversible manner. Indeed, Red(aq) can be also generated electrochemically from Ox(aq), under conditions of applied potential. We suppose that electroactive drug Red(aq) also undergoes a regenerative (chemically reversible) reaction with "Y" (present in large excess as dissolved species in solution). Chemical reaction between Red(aq) and Y(aq) regenerates the initial electroactive drug Ox(aq) and the substrate "S". We define "Y" and "S" as

"electrochemically inactive drugs", and we suppose that both do not show electrochemical activity in the window of applied potentials. We also assume that "S" and "Y" do not react between them. A limiting situation of considered catalytic reversible EC'rev mechanism is the catalytic irreversible EC'irr mechanism, obtained for *K*eq > 100 [24, 25]. At constant scan rate and for defined parameters related to the electron transfer step of electrode reaction (k_s^{Θ}, D and α), the cyclic voltammograms of this specific mechanism are function of equilibrium constant *K*eq, and of dimensionless catalytic parameter $K_{catalytic} = (k_f + k_b)\tau$. The last parameter is related to the rate of chemical reaction vs. the measuring time at potential steps. In scenario of large concentrations of "S" or "Y", the forward and backward rate constants of chemical reaction kf and kb are linked to the bulk substrates concentrations as: $k_f = k_f^{'}$ c(S) and kb = $k_b^{'}$ c(Y). This implies that in real experiments, $K_{catalytic}$ can be modified by altering molar concentrations of substrates S or Y.

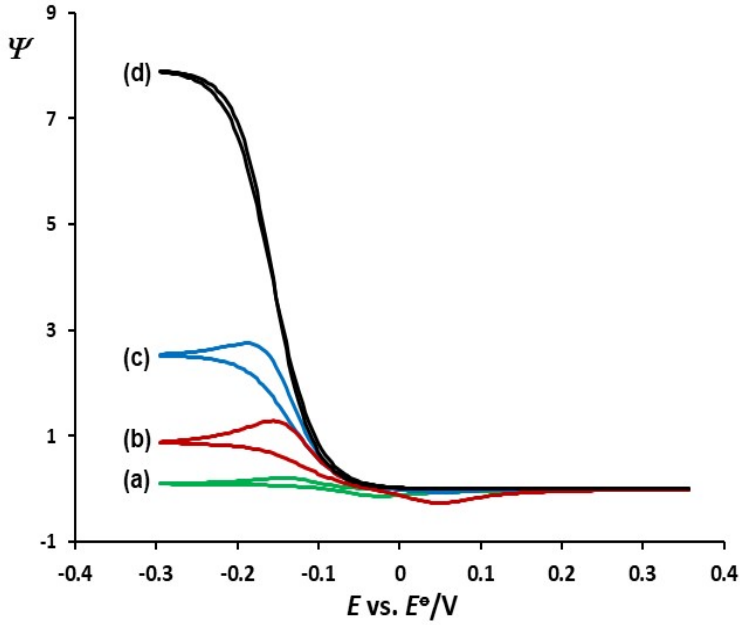


Figure 4. Cyclic voltammograms of diffusional catalytic EC'rev mechanism calculated for value of equilibrium constant *K*eq=1000, and for values of catalytic parameter $K_{catalytic}$=0.3 (a), 0.5 (b), 1 (c) and 100 (d). The value of dimensionless kinetic parameter of electrode reaction was set to K_{ET}=0.1. The other simulation parameters were same as in Figure 1.

Cyclic voltammograms calculated for K_{ET} = 0.1, *K*eq=1000, and for several values of $K_{catalytic}$ are presented in Figure 4. Voltammetric patterns of reversible EC'rev mechanism, simulated under such conditions, have features identical as of "simple" electrochemical catalytic EC' mechanism associated with irreversible chemical step [2, 5-7]. If $K_{catalytic}$ is small relative to current-measuring time-scale, observed cyclic voltammograms have quasi-reversible features, with the peak current ratio between the cathodic and anodic peaks ≈ 1

(voltammogram 1 at Figure 4). As the rate of catalytic reaction increases, we observe increasing in the current of the forward peak and a concomitant decrease of the backward currents. At large values of $K_{catalytic}$, significant amount of Ox will be chemically regenerated during the current-measuring time of given potential steps. This scenario will result in voltammetric shapes that get transition features between diffusion controlled and a steady-state behavior (curves 2-3 in Figure 4). Another feature of this mechanism is seen in the elevation of the "after-peaks" currents recorded at negative potentials. For $K_{catalytic} > 0.2$, the oxidation current branch gets lost, and both current components get identical sigmoidal shape that is typical for steady-state voltammograms (curve 4 in Figure 4). This happens when the rate of chemical regenerative reaction gets much faster than the rate of the re-oxidation electrode step. Under such circumstances, we get multiple occurrence of the $Ox + ne^- \rightarrow Red$ electrochemical reaction at all potentials. In such scenario, both current branches will be reduction currents, and the voltammetric patterns will get steady-state features [2, 5-14].

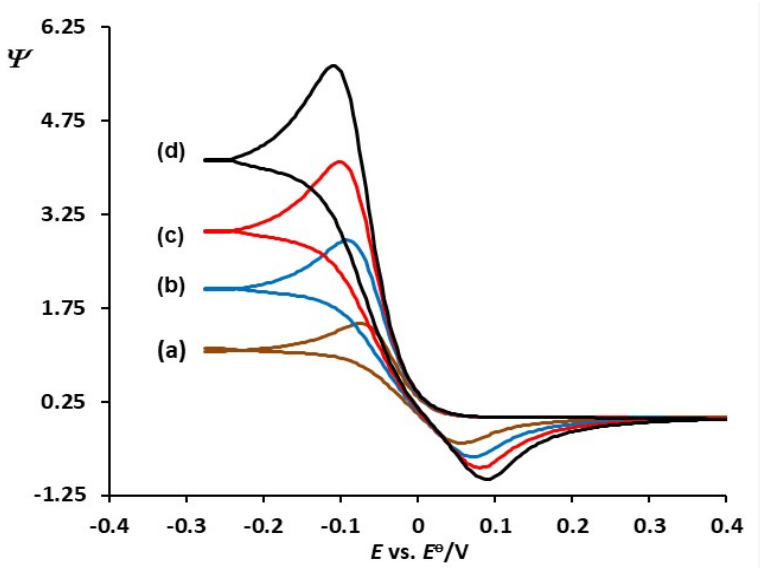


Figure 5. Cyclic voltammograms of diffusional catalytic EC'rev mechanism calculated as a function of equilibrium constant of regenerative chemical reaction *K*eq. *K*eq was set to: 0.1 (a); 0.0316 (b) 0.01 (c) and 0.0001 (d). Curves are simulated for value of catalytic parameter $K_{catalytic}$= 0.0316. The value of dimensionless kinetic parameter of electrode reaction was set to K_{ET} =0.5. Other simulation conditions were same as those in Figure 1.

Under conditions of steady-state voltammograms, the magnitude of limiting current ($I_{limiting}$) ("plateau" current) of cyclic steady-state voltammograms is affected only by the rate of regenerative chemical reaction and it is a linear function of $(K_{catalytic})^{0.5}$. The relationship between the $I_{limiting}$ and $(K_{catalytic})^{0.5}$ can be explored for quantitative purposes, but also for determination of the kinetics of drug-drug interactions as elaborated in [5-8, 11-14, 24, 25]. Other remarkable features of this catalytic EC' mechanism are recognized in the role of equilibrium constant of regenerative chemical step. As we know, the equilibrium constant of

regenerative step *K*eq determines the ratio of Ox and Red species available to undergo electrochemical transformation. Consequently, we expect that *K*eq should influence all relevant features of cyclic voltammograms. Effect of *K*eq to the cyclic voltammograms of this mechanism is illustrated in Figure 5. We can observe from patterns in Figure 5 that *K*eq affects all relevant parameters of the cyclic voltammograms, i.e. the height of the peaks, the peak-to-peak separation ($\Delta Ep = |(E_{p,c}-E_{p,a})|$), and the currents magnitudes measured at the negative potentials. For values of $K_{catalytic} > 0.2$ a regular shift of the half-wave potential $E_{1/2}$ in negative direction is observed. The dependence between $E_{1/2}$ and $K_{catalytic}$ can be explored for determination of standard rate constant of electron transfer, and for equilibrium constant *K*eq, as explained in [5-7, 25].

2.1.4. Surface CE, EC and EC' mechanism

The surface mechanisms coupled with chemical equilibria elaborated in this work are relevant to the voltammetric behavior of lipophilic drugs whose both redox forms (Ox and Red) are strongly adsorbed at the working electrode surface. Voltammetric patterns of all considered surface mechanisms have several specific features that can be shortly summarized in: a) sharper cathodic and anodic peaks than the corresponding peaks at diffusional mechanism; b) absence of diffusional tail; c) voltammetric features susceptible to lower chemical rates than by corresponding diffusional mechanisms; d) linear dependence of the limiting currents (in mechanisms 4 and 6) as a function of $K_{chemical}$.

The dimensionless kinetic parameter related to the electron transfer step at all surface mechanisms is defined as $K_{ET}=k_s^{\Theta}\tau$. The kinetic and thermodynamic parameters related to the chemical steps in all surface mechanisms are defined identically as at corresponding diffusional mechanisms. Since the chemical parameters $K_{chemical}$ and *K*eq cause similar effects to the voltammetric patterns as in the corresponding diffusional mechanisms 1-3, we present in Figures 6-8 only representative sets of simulated voltammograms of all considered surface mechanisms. In respect to the corresponding diffusional mechanisms, remarkable differences exist in the dependence of limiting currents $I_{limiting}$ at surface CE and surface EC' mechanisms. At surface CE and the EC' mechanism, the limiting currents $I_{limiting}$ of cyclic voltammograms (recorded in the kinetic regions) are proportional to $K_{chemical}$. Recall that at diffusional CE and EC' mechanisms, $I_{limiting}$ was linear function of $(K_{chemical})^{0.5}$. More comprehensive discussions about features of surface CE, EC and EC' mechanisms under conditions of cyclic voltammetry can be found in [2, 26]. In Table 1 we give data about the determination of relevant chemical parameters at all considered mechanisms, which can be used to characterize given drug-drug interactions. Moreover, in the same table we give short description about the specific voltammetric features that are caused by the rate of corresponding chemical reaction. At this, it is worth to mention once again that we assume in our models experiments performed at constant scan rates. In such scenarios, variation of chemical parameter $K_{chemical}$ can be achieved

via modification of concentration of substrate "Y", while keeping at constant value all other parameters.

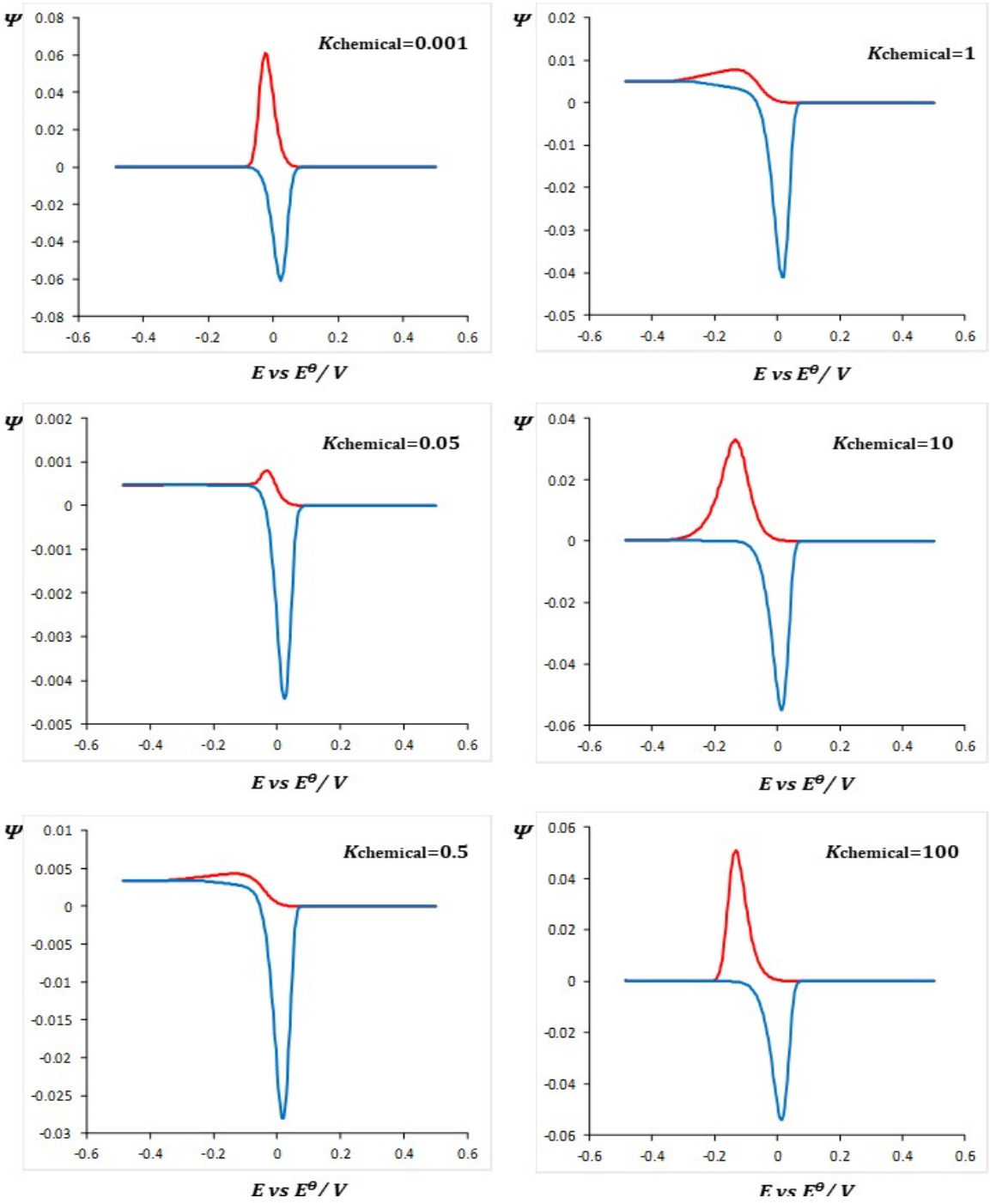


Figure 6. Influence of the chemical parameter $K_{chemical}$ to the features of cyclic voltammograms of a surface CE mechanism simulated for value of equilibrium constant Keq = 0.01. Value of dimensionless kinetic parameter of electron transfer was K_{ET}= 0.5. The values of chemical rate parameter $K_{chemical}$ are given in the charts. Other simulation conditions were same as those in Figure 1.

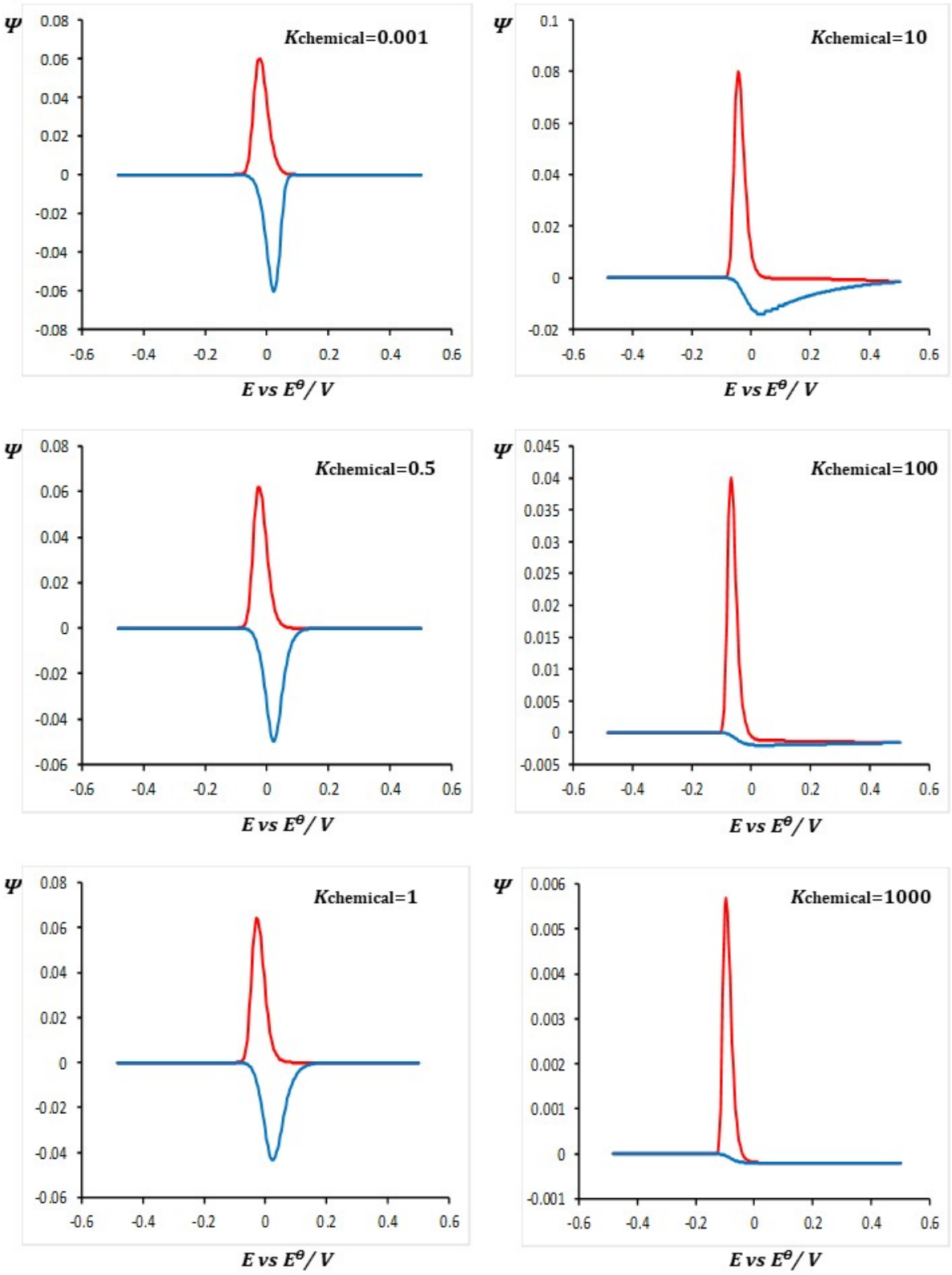


Figure 7. Influence of the chemical parameter $K_{chemical}$ to the features of cyclic voltammograms of a surface EC mechanism simulated for value of equilibrium constant Keq= 1. Value of dimensionless kinetic parameter of electron transfer was K_{ET}= 0.5. The values of chemical rate parameter $K_{chemical}$ are given in the charts. Other simulation conditions were same as those in Figure 1.

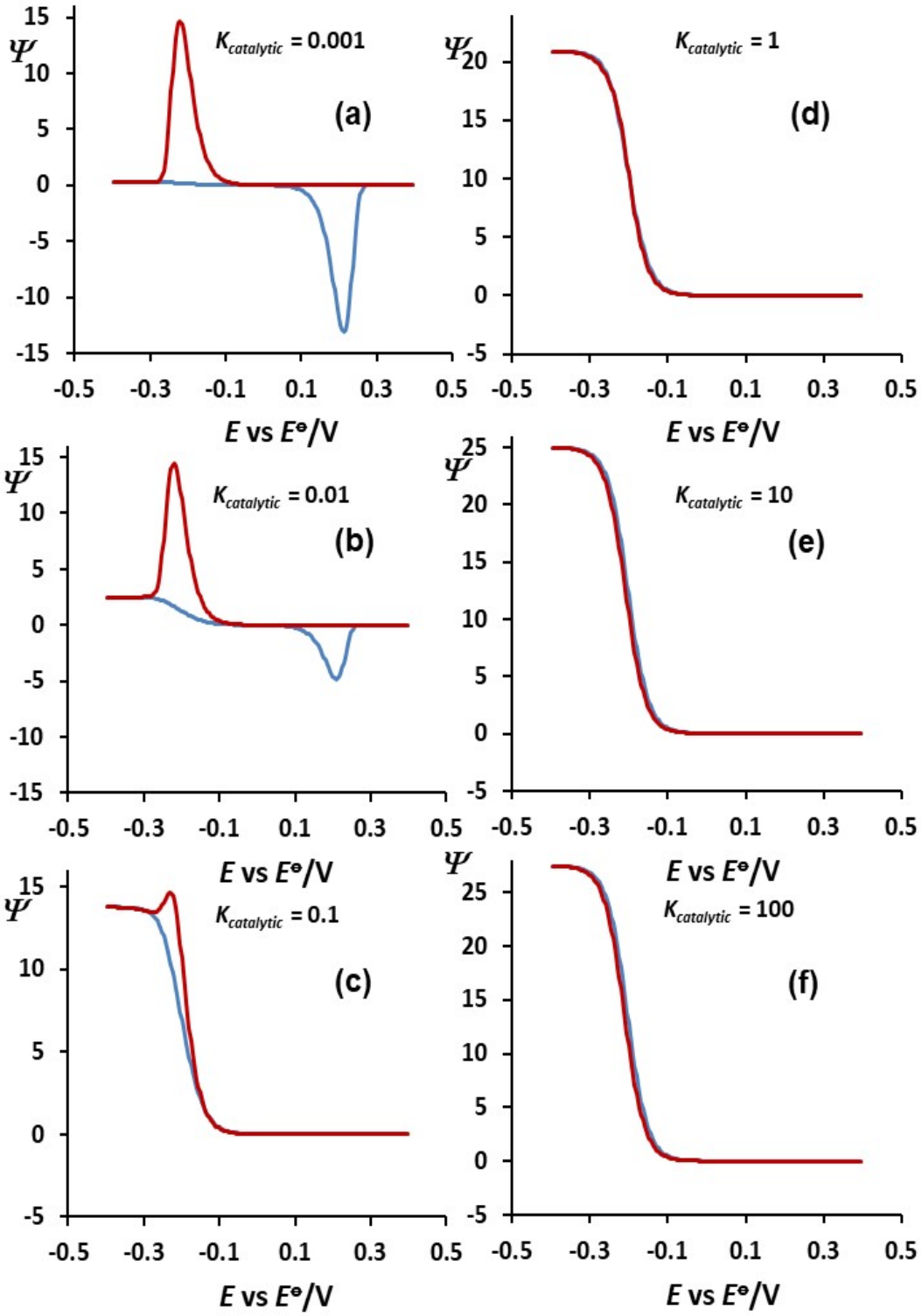


Figure 8. Influence of rate of chemical regenerative reaction to the features of theoretical cyclic voltammograms of a surface catalytic ECrev' mechanism. Voltammograms are simulated for six values of $K_{catalytic}$ (values are given in the charts) for K_{ET} of 0.1, and for K_{eq}= 1. The other parameters used in simulation model were identical as in figure 1.

Table 1. Description of the relevant voltammetric features at all considered mechanisms, and proposed methods for the determination of the kinetic and thermodynamic parameters related to the chemical steps

Type of mechanism	**Definition of dimensionless chemical rate parameter**	**Definition of equilibrium constant**	**Some characteristic effects to cyclic voltammograms caused by an increase of chemical rate parameter $K_{chemical}$**	**How to get access to *K*eq**	**How to get access to $K_{chemical}$**
Diffusional CE	$K_{chemical} = (k_f+k_b)\tau$	$K\text{eq}= k_f/k_b$	-plateau-like feature of forward currents in region of small values of *K*eq -shift of the mid-peak potential in negative direction for (-59 mV/n) for every tenfold increase of $K_{chemical}$ at systems with small and moderate value of *K*eq	-from the intercept of dependence $E_{mid,p}$ vs. $\log(K_{chemical})$ in kinetic region -via "two-parameter fitting procedure"	In the kinetic region, from the equation of limiting current $I_{limiting}$ that is defined as: $I_{limiting}= nFSc^*[K_{chemical}DK\text{eq}/\tau]^{0.5}$ (values of *K*eq, *D* and initial concentration of reactant c* should be known) -via "two-parameter fitting procedure"
Diffusional EC	$K_{chemical} = (k_f+k_b)\tau$	$K\text{eq}= k_f/k_b$	-permanent diminishment of backward peak current by increasing of Kchemical in the kinetic region -shift of the mid-peak potential in positive direction for (59 mV/n) for every tenfold increase of Kchemical at systems with small and moderate value of Keq	-from the intercept of linear dependence $E_{mid,p}$ vs. $\log(K_{chemical})$ in kinetic region -via "two-parameter fitting procedure"	From the $I_{p,a}/I_{p,c}$ curve constructed as a function of $\log(K_{chemical})$ -via "two-parameter fitting procedure"
Diffusional catalytic EC'rev	$K_{chemical} = (k_f+k_b)\tau$	$K\text{eq}= k_f/k_b$	-decrease of backward and concomitant increase of forward currents obtained by increasing of $K_{catalytic}$ -steady-state cyclic voltammograms featuring plateau are obtained at higher values of chemical rate parameter $K_{catalytic}$ -limiting current of cyclic voltammograms Ilimiting is a linear function of square-root of catalytic rate parameter $(Kcatalytic)^{0.5}$ -half-peak potential $E_{1/2}$ of steady-state voltammograms shifts in negative direction by increasing of $K_{catalytic}$	from the intercept of the mid-peak potential $E_{1/2}$ as a function of $\log(K_{catalytic})$, (provided that standard redox potential $E_{Ox/Red}$ is known)	From the equation of $I_{limiting}$ vs. $(K_{catalytic})^{0.5}$ linear dependence: $I_{limiting} =nFS[(c^*(Ox)(DK_{catalytic})^{0.5}]/(\tau)^{0.5}$ (initial concentration of Ox- c*(Ox) and diffusion coefficient *D* should be known)

Surface CE	$K_{chemical}$ = $(k_f+k_b)\tau$	Keq= k_f/k_b	-forward currents get shape of plateau in region of small values of K_{eq} by increasing $K_{chemical}$ - In the kinetic region, limiting current $I_{limiting}$ is proportional to $K_{chemical}$ -mid-peak potentials shift in negative direction for (-59 mV/n) for every tenfold increase of $K_{chemical}$ at systems with small and moderate value of Keq -absence of diffusional tail at cyclic voltammograms	from the intercept of dependence $E_{mid,p}$ vs. $\log(K_{chemical})$ in kinetic region	In the kinetic region, limiting current $I_{limiting}$ is defined as: $I_{limiting}= nFSI^*(K_{chemical}K\text{eq}/\tau)$ (one should know the values of Keq and I^* are known)
Surface EC	$K_{chemical}$ = $(k_f+k_b)\tau$	Keq= k_f/k_b	-backward currents diminish by increasing of $K_{chemical}$ -mid-peak potential shifts for (59 mV/n) for every tenfold increase of $K_{chemical}$ at systems with small and moderate value of Keq - ratio $I_{p,a}/I_{p,c}$ decreases by increasing of $K_{chemical}$ -absence of diffusional tail at cyclic voltammograms	-from the intercept of linear dependence $E_{mid,p}$ vs. $\log(K_{chemical})$ in kinetic region (if $E^{O}_{Ox/Red}$ is known) -via "two-parameter fitting procedure"	From the $I_{p,a}/I_{p,c}$ curve constructed as a function of $\log(K_{chemical})$ -via "two-parameter fitting procedure"
Surface catalytic EC'rev	$K_{chemical}$ = $(k_f+k_b)\tau$	Keq= k_f/k_b	-decrease of backward and concomitant increase of forward currents obtained by increasing of $K_{catalytic}$ -steady-state cyclic voltammograms featuring plateau are obtained at higher values of chemical rate parameter $K_{catalytic}$ -limiting current of cyclic voltammograms $I_{limiting}$ is a linear function of catalytic rate parameter $K_{catalytic}$ -half-peak potential $E_{1/2}$ of steady-state voltammograms shifts in negative direction by increasing of $K_{catalytic}$ -absence of "diffusional tail"	-from the intercept of the mid-peak potential $E_{1/2}$ as a function of $\log(K_{catalytic})$, (provided that standard redox potential $E_{Ox/Red}$ is known)	From the equation of $I_{limiting}$ vs. $(K_{catalytic})$ linear dependence: $I_{limiting}=nFS[I^*(\text{Ox})\ (K_{catalytic})/\tau$ [27] (initial surface concentration of Ox-I^*(Ox) should be known)

*S is the surface area of working electrode [cm^2]; F is Faraday constant (96500 C/mol); τ is duration of potential step in cyclic staircase voltammetry (s); I^*(Ox) is total surface concentration of Ox species [mol/cm^2]

2.2. Experimental voltammetric studies on drug-drug and drug-DNA interactions

In Table 2 we give experimental examples about some relevant chemical parameters of drug-drug and drug-DNA interactions evaluated by cyclic voltammetry. We focus on experimental works in this filed that are published mainly in last ten years.

Table 2. Experimental parameters (evaluated from cyclic voltammetry) related to stability constants of drug-drug and drug-DNA interactions

Drug-drug (or Drug-DNA)	**Experimental conditions**	**Equilibrium (stability) constant *K*eq**	**Ref.**
Novobiocin and cysteine	pH of 5.00; 7.00; and 9.00; HMD electrode	3.06×10^3 M^{-1} (in pH of 5.0) 1.06×10^5 M^{-1} (pH of 7.0) 1.06×10^5 M^{-1} (pH of 9.0)	[28]
Anthracycline calf thymus DNA	pH = 7.00; Glassy carbon electrode	4.44×10^5 M^{-1}	[29]
Ortho 2-(5-fluoro-2,4 dioxo-3,4-dihydroppyrimidin-1(2H)-yl)-N-(pyridin-2-yl) acetamide-DNA	pH = 7.16; Glassy carbon electrode	2.33×10^3 M^{-1}	[30]
Meta 2-(5-fluoro-2,4 dioxo-3,4-dihydroppyrimidin-1(2H)-yl)-N-(pyridin-2-yl) acetamide-DNA	pH = 7.16; Glassy carbon electrode	6.60×10^3 M^{-1}	[30]
flutamide (4-nitro-3-trifluoromethylisobutylanilide)-DNA	pH = 7.00; CPE (carbon paste electrode)	-	[31]
moxifloxacin (MOXI) in tablets-Cu(II) ion	pH = 8.00; HMDE	-	[32]
Omeprazole-azithromycin	pH = 7.40; Glassy carbon electrode	3.930 μM^{-1}	[33]
Omeprazole-clarithromycin	pH = 7.40; Glassy carbon electrode	0.985 μM^{-1}	[33]
Omeprazole- roxithromycin	pH = 7.40; Glassy carbon electrode	0.321 μM^{-1}	[33]
Omeprazole- doxycycline	pH = 7.40; Glassy Carbon electrode	8.230 μM^{-1}	[33]
Doxorubicin (DXH)-DNA	pH = 7.40; Glassy Carbon electrode	5.00×10^5 M^{-1}	[34]
Epirubicin (EpiDXH)-DNA	pH = 7.40; Glassy Carbon electrode	0.11×10^5 M^{-1}	[34]
Daunorubicin (DNR)-DNA	pH = 7.40; Glassy Carbon electrode	17.2×10^5 M^{-1}	[34]
Doxorubicin hydrochloride- calf thymus DNA	pH = 7.40; Glassy carbon electrode	1.05×10^5 M^{-1}	[35]
Acetaminophen (paracetamol) - antidepressant drugs ((a) fluoxetine, (b) sertraline and (c) nortriptyline)	pH = 7.0; Glassy carbon electrode	-	[36]
Cobalt(III) Schiff Base Complex [trans-$[Co(salen)(DA)_2]ClO_4$ -CT-DNA	pH=7.40; Glassy carbon electrode	1.31×10^4 M^{-1}	[37]
Cobalt(III) Schiff Base Complex trans-$[Co(salophen)(DA)_2]ClO_4$ (DA – dodecylamine)]- CT-DNA	pH=7.40; Glassy carbon electrode	3.15×10^4 M^{-1}	[37]
Dopamine (DA)- herring sperm deoxyribonucleic acid (ds-DNA)	pH = 7.00; Glassy carbon electrode modified with silver-doped Poly Cysteine membrane	1.08×10^5 M^{-1}	[38]
Epinephrine (Epi)-Fe^{3+}	pH = 7.40; Boron-doped diamond electrode	-	[39]
Epinephrine (Epi)-Fe^{2+}	pH = 7.40; Boron-doped diamond electrode	-	[39]
Cu(II) complex-DNA	Glassy carbon electrode	27.5×10^4 M^{-1}	[40]
Zn(II) complex-DNA	Glassy carbon electrode	64.5×10^4 M^{-1}	[40]
Ceftriaxone with phenylalanine	pH = 7.00 ; Glassy carbon electrode	1.32×10^3 M^{-1}	[41]
Pyrimethamine with dsDNA	pH = 7.00; Glassy carbon electrode	4×10^5 M^{-1}	[42]
$[CuL_2]Cl_2xH_2O$ where L= 1-amidino-O-ethylurea with DNA	pH = 7.20; Pt electrode	1×10^4 M^{-1}	[43]
Noglamycin with DNA	pH of 7.00; Glassy carbon electrode	4.44×10^5 M^{-1}	[44]
Ciprofloxacin with DNA	pH = 7.00; Glassy carbon electrode modified with MWCNTs+DNA	2.90×10^5 M^{-1}	[45]
Tyrosine with DNA	pH = 7.00; Glassy carbon electrode	3.98×10^3 M^{-1}	[46]
Sulfadiazine with DNA	pH = 7.00; Glassy carbon electrode modified with MWCNTs	2.87×10^3 M^{-1}	[47]

In most of the cases, a glassy carbon electrode, or carbon electrodes modified with nano-tubes are used as working electrodes. From the magnitudes of the stability constants in Table 2, we can see that experimentally determined *K*eq (or *K*stability) values related to elaborated drug-drug or drug-DNA complexes range between 10^3 and 10^6 M^{-1}. These values of *K*eq imply moderate stability of studied drug-drug or drug-DNA complexes. This means that cyclic voltammetry gives very good results for *K*eq for complexes with small and moderate values of stability constants [2], which is proven in many of the experimental works elaborated in this review.

3. CONCLUSION

We shortly elaborate in this review six theoretical models in cyclic voltammetry that are relevant to study drug-drug and drug-DNA interactions from many aspects. As we present large sets of simulated cyclic voltammograms related to defined mechanisms coupled to particular chemical reaction, we also give hints to evaluate thermodynamic and kinetics parameters relevant to drug-drug or drug-DNA interactions. Our advice to experimentalists working in this field is to perform voltammetric experiments related to drug-drug interactions at constant scan rate. Under such conditions, the rate of chemical reactions in all mechanisms can be modified by varying the molar concentration of substrate "Y" in voltammetric cell. As explained explicitly in the theoretical part of this mini-review, in order to evaluate kinetic and thermodynamic parameters relevant to drug-drug interactions, we must "work" in so-called "kinetically controlled regions" of cyclic voltammograms. At all elaborated mechanisms, we can achieve this scenario by adjusting the duration of potential step τ in CV and by varying the molar concentration of electrochemically inactive drug (substrate) "Y". In this way, we can create experimental conditions to reproduce the "kinetic" voltammetric patterns as those presented in figures 1 to 8. This will further allow estimation of $K_{chemical}$ and *K*eq from the equations or dependences described in Table 1. This review shows that cyclic voltammetry can be seen as a fast, simple and reliable tool that can provide insight into various aspects of drug-drug interactions. The major limitations of cyclic voltammetry come from complex adsorption phenomena and interfering compounds that commonly make complications at all electrode mechanisms.

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