Anal. Bioanal. Electrochem., Vol. 10, No. 5, 2018, 566-575

Analytical & Bioanalytical Electrochemistry

2018 by CEE
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Full Paper

A Time-Independent Approach to Evaluate the Kinetics of Enzyme-Substrate Reactions in Cyclic Staircase Voltammetry

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Received: 4 February 2018 / Received in revised form: 11 April 2018 / Accepted: 6 May 2018 / Published online: 31 May 2018

Abstract- In this work we refer to a simple and time-independent cyclovoltammetric approach for evaluating kinetics of chemical step of a diffusional electrocatalytic regenerative (EC') mechanism. The methodology relies on the dependence of the maximal catalytic (or limiting) current of the cyclic steady-state voltammograms on the catalyzing agent concentration. The magnitude of the limiting cyclovoltammetric current of an EC' mechanism depends exclusively on the rate of the chemical regenerative reaction, while it is independent on all kinetic and thermodynamic parameters related to the electrode reaction. Theoretical results of a quasireversible EC' mechanism reveal that the limiting current of calculated cyclic voltammograms is a linear function of the square-root of the dimensionless catalytic rate parameter. Because the rate of the catalytic reaction can experimentally be modified by altering the concentration of the regenerating agent (for constant scan rate), this scenario can be explored for the determination of the catalytic rate constant. The approach described in this work is important for the enzyme-substrate catalytic reactions, since the value of the catalytic rate constant allows estimation of the Michaelis-Menten constant.

Keywords- EC' mechanism, Catalytic rate constant, Cyclic voltammetry, Limiting currents, Quasi-reversible electrode reaction

1. INTRODUCTION

Cyclic voltammetry (CV) is a very popular electrochemical technique in identifying diverse electrode mechanisms [1-5]. It is considered as one of the most advanced

electrochemical techniques for getting insight into the mechanistic pathways of various electrochemical reactions. Alongside, CV is a powerful technique for estimating the kinetics and thermodynamics of electrode reactions, but it also provides access to physical parameters relevant to the chemical reactions coupled to electrode reactions [1-16]. Among the systems studied with cyclic voltammetry, those involving chemical reactions coupled to the electron transfer steps are of great significance as they mimic important biological reactions [1,17-20].

Although cyclic staircase voltammetry is considered as a very similar with the linear cyclic voltammetry, these two techniques differ in one very crucial aspect. While the wave form in linear cyclic voltammetry is a linear ramp, a staircase ramp featuring small equivalent potential jumps (called a "potential step") is a wave form in the cyclic staircase voltammetry. This gives the cyclic staircase voltammetry significant advantage over the linear cyclic voltammetry in the discrimination against capacitance currents. Both techniques can give similar results if a very small potential step (approximately<0.1 mV) is used in cyclic staircase voltammetry. The electrocatalytic-regenerative mechanism (or simply, EC' mechanism) is one of the most thoroughly elaborated mechanisms in cyclic voltammetry [1,3,4,6,9-16,18,19,22-27]. Although it has been intensively studied for over 50 years [28], the EC' mechanism is still a subject of interest by many electrochemists. Main reason educes from the fact that this mechanism is present in bioorganic electrochemistry, especially in the enzymatic reactions [9,17,19,20,29]. In this reaction mechanism, the electron transfer step (E) taking place at the working electrode surface is coupled to a subsequent chemical reaction (C'). The chemical reaction of the EC'mechanism contributes to regeneration of the starting electroactive compound of the electrode reaction. This is achieved via a following redox reaction between the final product of the electrode reaction and a given catalyzing agent that is dissolved in electrochemical cell. Various aspects of the EC' mechanism are published in many papers so far [1,3,4,6-12,15-19,21,22-24]. We recently published a theoretical work, while referring to several new aspects of the EC' mechanism under conditions of square-wave voltammetry [9]. In [9], we provided a new time-independent square-wave voltammetric approach to access the kinetics of electron transfer step of electrode reaction. An analytical solution for evaluating the kinetics of chemical step of a reversible electrode reaction of EC' mechanism in linear cyclic voltammetry is given in [1,17]. In this work we present a simple time-independent method to access kinetics of catalytic reaction of an EC' mechanism featuring quasi-reversible electrode reaction under conditions of cyclic staircase voltammetry.

2. MATERIALS AND METHODS

In the theoretical calculations, we considered an electrochemical-catalytic (regenerative EC') mechanism under conditions of cyclic staircase voltammetry. All species in the

electrochemical cell are present in dissolved state and no adsorption phenomena take place at the working electrode. It is considered that the mass transfer of all species to the working electrode surface occurs via semi-infinite planar diffusion. We used MATHCAD 14 software for performing all calculations. A detailed description of the algorithm used is given in [9].

3. RESULTS AND DISCUSSION

We consider theoretically an electrode mechanism, in which the product Red of the electrode reaction Ox+ne-↔Red, undergoes a chemical reaction with dissolved non-electroactive species S. The occurrence of chemical reaction between Red and S contributes to regeneration of the starting material Ox at the electrode surface:

Electrochemical (E) step: $Ox + ne- \overset{k}{\rightleftharpoons} Red$

Catalytic (C') step: $Red + S \xrightarrow{k_c} Ox$ (+ side products)

All the species Ox, Red and S, are present in dissolved state and no adsorption phenomena take place at the working electrode. At the beginning of the experiment, only species Ox are present in the electrochemical cell. In the theoretical model, we consider a quasi-reversible electrode reaction Ox+ne-↔Red that is coupled with irreversible regenerative chemical reaction under conditions of cyclic staircase voltammetry. The mass transfer at the working electrode surface takes place via semi-infinite planar diffusion. It is worth mentioning that recent potentiostats use almost exclusively staircase wave form of the potential in cyclic voltammetry, so the cyclic "staircase" voltammetry is used as an alternative technique for linear cyclic voltammetry. More details about the simulation procedure and the algorithm used can be found elsewhere [9,19,29]. Calculated voltammograms of a diffusional EC' mechanism depend on several physical parameters, i.e. the thermodynamic temperature T (it was set at 298 K); the potential step of the staircase ramp dE (it was set to 10 mV), the number of electrons exchanged-n; the diffusion coefficient D (it was assumed that the diffusion coefficients of both Ox and Red were equal and set to 3×10^{-6} cm^2s^{-1}), and the electron transfer coefficient α. Moreover, the main features of simulated cyclic voltammograms are mainly affected by two dimensionless kinetics parameters λ and γ. The kinetics of the electrode reaction is portrayed via the magnitude of the dimensionless kinetic parameter-λ, defined as $\lambda=k_s\tau^{0.5}D^{-0.5}$, where k_s is the standard rate constant of electron transfer, and τ is the duration of the potential step of the signal applied. The kinetics of the chemical step (C') is revealed via the magnitude of dimensionless catalytic parameter-γ ($\gamma=k_c\tau$), where k_c is the rate constant of the catalytic reaction. While the dimensionless kinetic parameter λ represents the apparent electrochemical reversibility of the

electrode reaction (E), the dimensionless catalytic parameter γ portrays the influence of the kinetics of chemical regenerative reaction (C'). The dimensionless current of the simulated voltammograms Ψ is defined as: $\Psi=I[nFAc^*(\mathrm{Ox})]^{-1}\tau^{0.5}D^{-0.5}$, where I is the current (Amperes), F is the Faraday constant (96485 C/mol), A is the working electrode surface area (cm^2), and $c^*_{(Ox)}$ is the bulk concentration of Ox species. It is worth mentioning that k_c is a pseudo-first order rate constant of the catalytic reaction (C') defined as $k_c=k_{cat}c(S)$. Here, k_{cat} is the real rate constant of the chemical regenerative reaction ($mol^{-1}s^{-1}L$), while $c(S)$ is the molar concentration of the regenerating agent S. We defined in the model the cathodic currents as "negative".

In absence of regenerating species S, the electrochemically active species Ox and Red interconvert in accordance to the potential applied (curve 1, figure 1). However, when S is added in the electrochemical cell (curves 2-6, figure 1), then S starts reacting with Red in a way of chemical regenerating the Ox species. As Ox gets chemically regenerated, this extra portion of Ox material can undergo additional reduction at the working electrode in the current-measuring time window. This phenomenon leads to the increasing of the cathodic currents measured in the potential regions where the rate of the catalytic regenerative reaction affects the features of calculated cyclic voltammograms (curve 2 at figure 2). Roughly, this effect starts to be "sensed" at potentials of about +80 mV of the standard redox potential of Ox/Red and continues at further negative potentials. As the rate of the catalytic reaction increases, the anodic (oxidative) currents of the cyclic voltammograms start changing their sign. At a given high rate of the regenerative catalytic reaction, both currents branches of the cyclic voltammograms become "cathodic", having magnitudes very close to each other (curves 3 and 4 at figure 1). In such scenario, the kinetics of the chemical regenerative reaction S+Red→Ox becomes higher than the kinetics of the electrode reaction Red–ne-→Ox. This phenomenon will lead to a multiple repetitive occurrence of the electrode reaction Ox+ne-→Red at potentials applied. Eventually, the oxidation branch of the cyclic voltammograms merges completely with the reduction one (curves 5-6 at figure 1). This happens when the rate of the chemical catalytic reaction S+Red →Ox dominates significantly over the kinetics of the electrode reaction Red – ne-→Ox, approximately when the ratio $(\gamma/\lambda)>0.7$. Accordingly, under such circumstances, the entire amount of the Red species created at the working electrode surface, gets very quickly chemically converted into Ox species, and no diffusion layer exists. We obtain in this case a "steady-state" sigmoidal-shaped voltammograms (curves 5 and 6, figure 1) due to the exclusive occurrence of the electrode reaction Ox+ne-→Red at all applied potentials. The observable criterion for steady-state voltammograms is that by decreasing of the scan rate we see no effect on the "catalytic" voltammograms. In most of the cyclovoltammetric approaches elaborated so far, the voltammetric features like: ratio of peak-currents between the cathodic and anodic peaks; the peak-to-peak separation between the cathodic and anodic peak, as well as the mid-peak

potential shift, all regarded as function of the applied scan rates, have been explored as features for the determination of the rate constant of the catalytic reaction [1-8,18-24]. However, all these features evaluated from the cyclic voltammograms contain a sort of mixed nature, since they are affected not only of the kinetic constant of the catalytic reaction, but also to other parameters related to the electron transfer step of the electrode reaction [1-5,14,15,18-24]. In order to get exclusive access to the rate constant of the catalytic chemical reaction, the best approach is if we rely to some features of the cyclic voltammograms that are independent on physical parameters affecting the electrode reaction, but depend on the rate of the catalytic reaction only. Shown in figures 2 to 4 are cyclic voltammograms calculated for various values of the kinetics of the electron transfer step λ (figure 2), the electron transfer coefficient α (figure 3) and the number of electrons exchanged n (figure 4). All the voltammograms are simulated for a constant rate of the catalytic reaction (γ=1.256), at constant temperature and at constant scan rate. Obviously, the features of the simulated steady-state voltammograms in figures 2 to 4 are function of λ, α and n at, or nearby potentials to the value of the standard redox potential of redox couple Ox/Red. However, the maximal current magnitudes of the cyclic voltammograms (the so-called "catalytic plateau" or "limiting currents" of the cyclic voltammograms), measured at the potentials more negative of about ~200 mV than the standard redox potential of Ox/Red, are independent on λ, α and n.

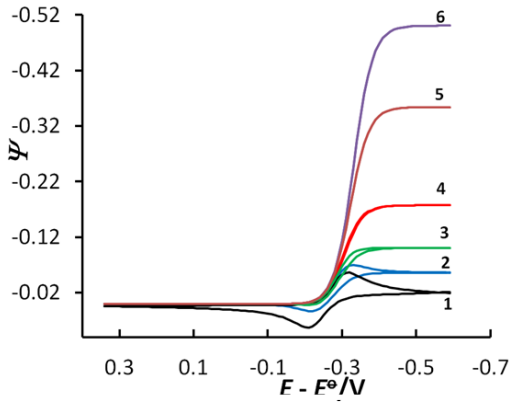

Fig. 1. Effect of the catalytic parameter γ on the features of the calculated cyclic staircase voltammograms simulated under following conditions: temperature T=298 K; dimensionless electrode kinetic parameter λ=0.032; diffusion coefficient D=3×10^{-6}; cm^{2}s^{-1}; number of electrons ne-=2, electron transfer coefficient α=0.5. The values of the dimensionless catalytic parameter were: γ=0.000032 (1), 0.001 (2), 0.0032 (3), 0.032 (4), 0.126 (5) and 0.251 (6)

In the case of very asymmetrical electron transfer barrier, the limiting currents should be measured at potentials of even 400 mV more negative than the standard redox potential of Ox/Red. The magnitude of the maximal catalytic currents of the steady-state cyclic

voltammograms depends mainly on the rate of the chemical regenerative reaction (see encircled regions of the voltammograms in figures 2, 3 and 4). In the region of very negative potentials, the kinetics of the electron transfer step of electrode reaction is, indeed, very high. Consequently, the entire Ox material present in the proximity of the electrode surface will be very quickly reduced to Red at the working electrode. Therefore, the magnitude of the current in this potential region depends exclusively on the rate by which the catalytic step (the chemical reaction between Red and S) re-supplies Ox to the electrode surface. Experimentally, the rate of the catalytic reaction (under constant scan rate and constant temperature) can be altered by increasing the concentration of the catalyzing agent S in the electrochemical cell. Shown in figure 5 is the dependence of the limiting currents of the simulated cyclic voltammograms as a function of the square-root of the dimensionless catalytic parameter γ.

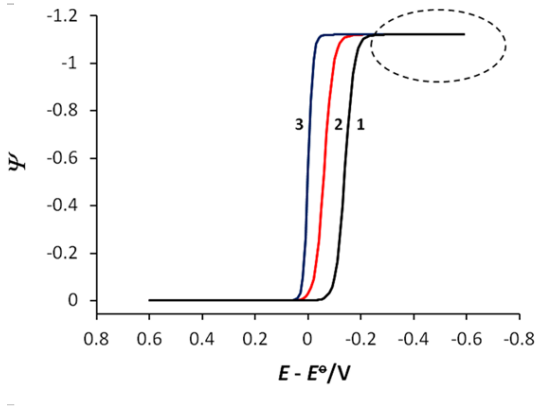

Fig. 2. Effect of the kinetics of the electrode reaction (λ) on the features of cyclic voltammograms, calculated for γ=1.256, and λ=0.000032 (1); 0.032 (2) and 32 (3). All the other simulation parameters were the same as those in figure 1

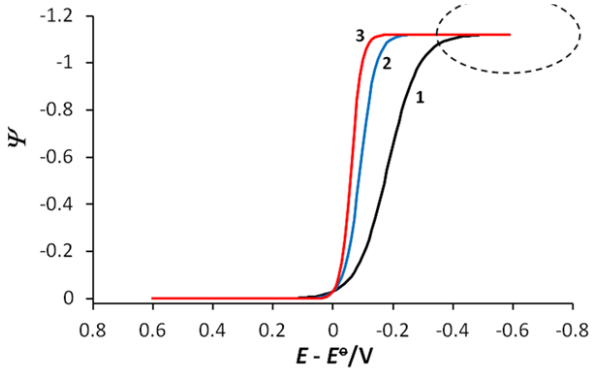

Fig. 3. Effect of the number of exchanged electrons in electrode reaction on the features of the cyllic voltammograms calculated for γ =1.256, λ=0.032, and *n*e-=1 (1); 2 (2) and 3 (3). All the other simulation parameters were the same as those in figure 1

The slope of the linear dependence between the Ψmax and $\gamma^{0.5}$ (R^2=1) allows direct access of the real value of the catalytic rate constant (k_{cat}) by exploring the equation corresponding to the linear dependence as that in figure 5.

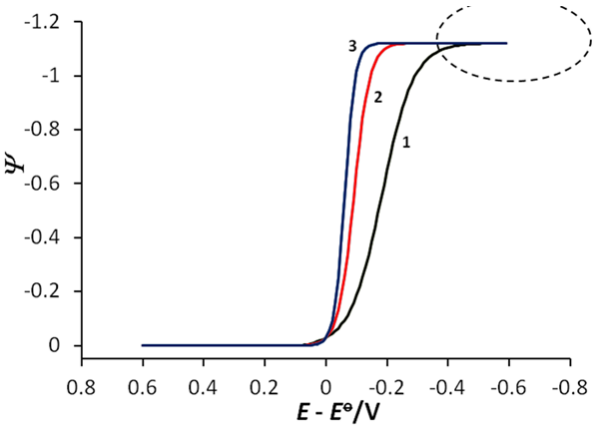

Fig. 4. Effect of the electron transfer coefficient of the electrode reaction on the features of the cyllic voltammograms calculated for γ=1.256, λ=0.032, and α=0.25 (1); 0.5 (2) and 0.75 (3). All the other simulation parameters were the same as those in figure 1

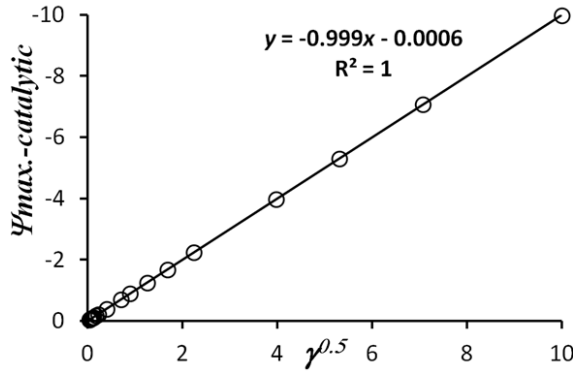

Fig. 5. Dependence of the maximal "plateau" catalytic currents of simulated cyclic voltammograms on the square root of the dimensionless catalytic parameter γ. All the other simulation parameters were the same as those in figure 1

4. CONCLUSION

Although the electrochemical-catalytic (EC') mechanism is one of the most elaborated under voltammetric conditions, new approaches for the kinetic and thermodynamic determinations at this mechanism still emerge. Majority of the voltammetric methods developed for the kinetic and thermodynamic evaluations of the EC' mechanism are either time-dependent or potential-dependent [1,3,4,6-8,14-19,30-33]. Only recently, couple of new

papers appeared, referring to alternative square-wave voltammetric methods for the voltammetric determination of rate constants of the electrode reactions [9,34-36]. In this work, we present a simple and time-independent cyclovoltammetric approach for evaluating the kinetic rate constant of the catalytic reaction of an EC' mechanism featuring quasi-reversible electrode reaction. Experimentally, the rate of the catalytic reaction can be modified either by altering the scan rate (under constant concentration of regenerating agent S), or by altering the concentration of S (under constant scan rate). Recalling the discussion for the figures 2 to 4 in this work, we understood that the magnitude of the limiting current of catalytic steady-state cyclic voltammograms depends exclusively on the rate of chemical regenerative reaction. The maximal catalytic current of the steady-state cyclic voltammograms is linear function of the square-root of the dimensionless catalytic parameter γ (see figure 5). Since the catalytic parameter γ is defined as $\gamma=k_c\tau$, and $k_c=k_{cat}c(S)$, we can reconstruct experimentally the theoretical dependence from the figure 5 in terms of varying the concentration of catalyzing agent $c(S)$. By plotting the limiting catalytic current magnitudes I_{max} of the plateau's of experimental cyclic voltammograms as a function of the square-root of the molar concentration of the catalyzing agent-$[c(S)]^{0.5}$, we recreate the dependence from figure 5. The slope of linear dependence I_{max} *vs.* $[c(S)]^{0.5}$ is defined as: $\boldsymbol{slope=\left|(k_{cat})^{0.5}[nFAc^{*}(Ox)\tau^{-0.5}D^{0.5}]\right.}$. Hence, if we know the number of electrons exchanged n and the value of the diffusion coefficient D for the non-catalyzed simple Ox+ne-↔Red system [for this, see 1-3,18], and if we work under constant scan rate (then we know τ), it becomes quite easy to get the value of k_{cat}. To achieve this, we do not need to know the magnitudes of the parameters related to the quasi-reversible electrode reaction (i.e. the standard rate constant of the electron transfer k_s, and the electron transfer coefficient α). Note that finding, evaluated for an EC' mechanism featuring quasi-reversible electrode reaction, is complementary with the analytical solution of the limiting cyclovoltammetric currents of an EC' mechanism featuring thermodynamically reversible electrode reaction, given in [1,17]. It is worth to mention that this approach can not be explored to the "net" responses recorded in square-wave voltammetry (SWV) due to the specific current sampling manner in SWV [23,30]. The determination of the catalytic rate constant k_{cat} is quite important for the enzymatic systems, since it allows access to the magnitude of the Michaelis-Menten constant. Michaelis-Menten constant is, indeed, a major kinetic parameter relevant to all enzyme-substrate reactions. This method allows estimation of the catalytic rate constants that fall in the region $\log(\gamma)>-3$. In our concurrent work, we will soon report on similar phenomena of the "protein-film cyclic voltammetry" of EC' systems.

Acknowledgments

Rubin Gulaboski thanks the "Goce Delcev" University in Stip, Macedonia, for the support.

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