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Article

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Reciprocal Sum Expression for Steady-state Kinetics -Enzyme Reactions and Voltammetry-

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ABSTRACT

Steady-state experiments are often conducted to understand complicated cases in chemistry, since the kinetics does not have a time valuable and allows simple modeling of the reactions. The reciprocal of the overall rate of sequential steady-state reactions is often given in the reciprocal sum formula: sum of the reciprocals of the rates of the hypothetical rate-limiting processes at the individual stages. In this paper, the reciprocal sum relationship is generalized for sequential multi-step steady-state reactions, and the importance and usefulness of the concept is shown by applying it to describe several typical steady-state systems in enzyme reactions and voltammetry using rotating disk- and ultramicro-electrodes.



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1. Introduction

Steady-state experiments are often conducted in several complicated cases in chemistry. Such steady-states are realized after presteady transient states, but the pre-steady interval may be shortened under well-designed conditions, for example, in enzyme reactions and steady-state voltammetry using rotating disk electrodes (RDEs) and ultramicroelectrodes (UMEs). Steady-state kinetics does not have a time valuable, and then the kinetic valuables can be measured with much higher accuracy than transient ones and kinetic models may be considered more easily and simply than in the case of transient systems.

The steady-state kinetics tells us little about the nature of intermediates, but provides us valuable information about the reaction rates of several reaction steps with rather simple equations.

Therefore, it is easy to focus on the kinetics of complicated reactions and to discuss them in detail. In steady-state kinetics, the reciprocal sum formulas, in which the reciprocal of the entire sequential reaction rate is given by the sum of the reciprocals of the rates of the hypothetical rate-limiting processes at the individual stages, are often used, as exemplified by the description of steady-state enzyme kinetics1 and steady-state voltammogram.2,3 Each term in the reciprocal sum formula should correspond to the hypothetical limiting value as a rate-determining step (rds).

In this paper, we will generalize the reciprocal sum relationship for sequential multi-step steady-state reactions and show the importance and usefulness of the concept by applying it to describe several typical steady-state systems in enzyme reactions and voltammograms at RDEs and UMEs.

2. Steady-State Reaction Rate in Sequential Reactions

First, for simplicity, let us consider a two-step mono-directional sequential steady-state reaction consisting of the prior reaction 1 and the subsequent reaction 2:

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$$\sum v_i \operatorname{Reac}_i \xrightarrow{\operatorname{reaction } 1} \sum v_j \operatorname{Int}_j \xrightarrow{\operatorname{reaction } 2} \sum v_k \operatorname{Prod}_k,$$
(1)

where Reac_i, Int_j, and Prod_k are the reactants, intermediates, and products, respectively. v_i (v_j , v_k) is the stoichiometric coefficient of Reac_i (Int_j, Prod_k). Under the steady-state conditions, the concentrations of the intermediates remain unchanged ($dc_j/dt = 0$; c_j and t being the concentration of Int_j and time, respectively) and then the Gibbs free energy change of the entire reaction system (dG) is given by:

$$\mathrm{d}G = \sum \mu_i \mathrm{d}n_i + \sum \mu_k \mathrm{d}n_k = \left(-\sum \mu_i \nu_i + \sum \mu_k \nu_k\right) \mathrm{d}\xi, \quad (2)$$

where μ_i (μ_k) and n_i (n_k) are the chemical potential and the amount of substance of Reac_i (Prod_k), respectively, and ξ is the extent of the reaction. The steady-state reaction rate expressed as $v_s = d\xi/dt$ is constant under steady-state conditions. Therefore, the rate of the Gibbs free energy change of the entire reaction system is constant:

$$\frac{\mathrm{d}G}{\mathrm{d}t}\left(=\left(-\sum \mu_i \nu_i + \sum \mu_k \nu_k\right)\frac{\mathrm{d}\xi}{\mathrm{d}t}\right) \equiv R\nu_{\mathrm{s}} = \mathrm{const.},\quad(3)$$

where *R* is called the reaction resistance of the total process. In a similar manner, we can define the reaction resistances (R_1 and R_2) for reactions 1 and 2, respectively:

$$\frac{\mathrm{d}G_1}{\mathrm{d}t} = R_1 v_{\mathrm{s}},\tag{4}$$

$$\frac{\mathrm{d}G_2}{\mathrm{d}t} = R_2 v_\mathrm{s}.\tag{5}$$

where G_1 and G_2 are the Gibbs energies of reactions 1 and 2, respectively. Considering the situation that $G = G_1 + G_2$ and substituting Eqs. 4 and 5 into Eq. 3, we can get the following relation on the reaction resistance:

$$R = R_1 + R_2. (6)$$

The sum concept is identical to that of the series resistance in the electric circuit. When reaction 1 is the hypothetical rate determining step (rds: $R_1 \gg R_2$, and thus $R_1 \rightarrow R$), the limiting steady-state reaction rate of reaction 1 ($v_{rds,1}$) is defined as

$$R_1 v_{\mathrm{rds},1} \equiv \lim_{R_1/R \to 1} R_1 v_{\mathrm{s}} = R v_{\mathrm{s}} = \frac{\mathrm{d}G}{\mathrm{d}t}.$$
 (7)

Similarly, when reaction 2 is an rds, the limiting steady-state reaction rate of reaction 2 ($v_{rds,2}$) is defined as:

$$R_2 v_{\rm rds,2} \equiv \lim_{R_2/R \to 1} R_2 v_{\rm s} = R v_{\rm s} = \frac{\mathrm{d}G}{\mathrm{d}t}.$$
 (8)

Substituting Eqs. 7 and 8 into Eq. 6, we obtain the following reciprocal sum relation:

$$\frac{1}{v_{\rm s}} = \frac{1}{v_{\rm rds,1}} + \frac{1}{v_{\rm rds,2}}.$$
(9)

In general, for an *n*-step sequential steady-state reaction, we may extend Eq. 9 to the following general reciprocal sum formula:

$$\frac{1}{v_{\rm s}} = \sum_{1}^{n} \frac{1}{v_{\rm rds,m}}.$$
(10)

The reciprocal of the steady-state rate of the reaction composed of nstep sequential reactions is given by the sum of the reciprocals of the steady-state limiting rates of the individual n rdss.

Here, we will consider an *n*-step sequential pseudo-first-order reaction:

$$\mathbf{A} \xrightarrow{\text{reaction 1}} \mathbf{B} \xrightarrow{\text{reaction 2}} \mathbf{C} \cdots \xrightarrow{\text{reaction } n} \mathbf{X}.$$
(11)

When reaction 1 is an rds, the concentration of A (c_A) is considered to be hypothetically identical with the initial total concentration of the reactant (c_0), and the hypothetical limiting rate of reaction 1 ($v_{rds,1}$) can be expressed as:

$$v_{\mathrm{rds},1} \equiv \lim_{c_1 \to c_2} k_1 c_{\mathrm{A}} = k_1 c_0,\tag{12}$$

where k_1 is the pseudo-first-order reaction rate constant of the 1st step. In a similar way, the limiting rate of the *m*-th step is given by:

$$v_{\mathrm{rds},m} \equiv \lim_{c_{\mathrm{M}} \to c_{0}} k_{m} c_{\mathrm{M}} = k_{m} c_{0},\tag{13}$$

where k_m is the pseudo-first-order reaction rate constant of the *m*-th step. Finally, the following general reciprocal sum formula for the pseudo-first-order reaction rate constant (*k*) of the sequential reaction can be obtained by substituting Eq. 13 to Eq. 10:

$$\frac{1}{k} = \sum_{1}^{n} \frac{1}{k_m}.$$
(14)

The reciprocal of the pseudo-first-order rate constants of the reaction composed of n-step sequential reactions is given by the sum of the reciprocals of the pseudo-first-order rate constants of the individual n rdss.

Next, let us consider the case where the 1st step reaction is bidirectional. For the simplicity, the following first-order reaction is considered as an example:

$$A \xrightarrow[k_{-1}]{k_{-1}} B \xrightarrow[k_{2}]{k_{-1}} C.$$
(15)

The overall reaction is composed of two-step reactions and has two rdss. When the 1st step is an rds, the hypothetical limiting steady-state reaction rate $(v_{rds,1})$ is given by:

$$v_{\rm rds,1} \equiv \lim_{c_{\rm A,s} \to c_0} k_2 c_{\rm B,s} = \lim_{c_{\rm A,s} \to c_0} k_2 c_{\rm A,s} \frac{c_{\rm B,s}}{c_{\rm A,s}} = k_2 c_0 K_{\rm s}, \quad (16)$$

where $c_{i,s}$ is the steady-state concentration of component *i*, and the steady-state concentration quotient (K_s) is defined by:

$$K_{\rm s} \equiv \frac{c_{\rm B,s}}{c_{\rm A,s}}.\tag{17}$$

When we use steady-state approximation for intermediate B ($dc_{B,s}/dt = k_1c_{A,s} - (k_{-1} + k_2)c_{B,s} = 0$), K_s is given as $k_1/(k_{-1} + k_2)$. However, if we use precursory rapid equilibrium approximation, K_s is given as k_1/k_{-1} .

When the 2nd step is an rds, the hypothetical limiting steady-state reaction rate ($v_{rds,2}$) is given by:

$$v_{\rm rds,2} = \lim_{c_{\rm B,s} \to c_0} k_2 c_{\rm B,s} = k_2 c_0.$$
(18)

The steady-state reaction rate of the entire reaction is given by:

$$v_{\rm s} = kc_0,\tag{19}$$

where k is the pseudo-first-order reaction rate constant of the entire reaction. Substituting Eqs. 16, 18, and 19 into Eq. 10, we obtain the following reciprocal sum formula:

$$\frac{1}{k} = \frac{1}{k_2 K_8} + \frac{1}{k_2}.$$
(20)

It is very useful to consider steady-state reactions in terms of the reciprocal of the pseudo-first-order rate constant. The kinetics of any of complex reactions can be expressed by reciprocal sum formulas such as Eqs. 10, 14, and 20 under steady-state conditions. Once one can understand the concept, it is very easy to derive kinetic equations for objective reactions. In addition, the reciprocal sum concept gives clearer pictures of the rdss; each term in the reciprocal sum formula corresponds to the kinetics of the individual rdss. Such a reciprocal sum formula was introduced as a harmonic sum.³ However, it is not a right term in mathematical sense, but it should be called reciprocal sum, as described here. In the following, we will

apply the reciprocal sum concept to some typical examples of enzyme kinetics and steady-state voltammetry.

3. Steady-State Enzyme Kinetics

3.1 Uni Uni mechanism

3.1.1 Michaelis-Menten equation

Enzyme reactions are often explained by the following simplified scheme, as shown in Eq. 21; a free enzyme (E) binds a substrate (S) to form an ES complex, which releases a product (P) to regenerate E:

$$\begin{array}{c} +S \\ E \xrightarrow{k_1 \ (=k_{1(2)}c_S)} \\ \hline k_{-1} \\ \end{array} \xrightarrow{k_2} E, \qquad (21) \\ S \end{array}$$

where k_1 is the pseudo-first-order reaction rate constant of the ES formation ($k_{1(2)}$ being the second-order reaction rate constant), while k_{-1} and k_2 are the first-order reaction rate constants of the corresponding processes. The steady-state reaction rate in the overall sequential reaction is identical with that of any of the monodirectional processes, and then $v_s = k_2 c_{\text{ES},s}$ ($c_{\text{ES},s}$ being the steady-state concentration of the ES complex) in the scheme of Eq. 21. Therefore, the hypothetical limiting steady-state rate ($v_{\text{rds},1}$) of the reaction in which the 1st step is an rds is given by:

$$v_{\mathrm{rds},1} \equiv \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 c_{\mathrm{ES},s} = \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 \frac{c_{\mathrm{ES},s}}{c_{\mathrm{E},s}} c_{\mathrm{E},s}$$
$$= \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 c_{\mathrm{E},s} \frac{c_{\mathrm{S}}}{K_{\mathrm{M}}} = k_2 c_{\mathrm{E}} \frac{c_{\mathrm{S}}}{K_{\mathrm{M}}}, \tag{22}$$

where $c_{\rm E}$ is the total concentration of E, and we define the Michaelis constant ($K_{\rm M}$) (as a steady-state concentration quotient) according to the nomenclature of the biochemistry field:

$$K_{\rm M} \equiv \frac{c_{\rm E,s} c_{\rm S}}{c_{\rm ES,s}} \,. \tag{23}$$

 $K_{\rm M}$ is expressed as $(k_{-1} + k_2)/k_{1(2)}$ in the steady-state approximation but as $k_{-1}/k_{1(2)}$ in the rapid equilibrium approximation. When the 2nd step is an rds, the hypothetical limiting steady-state reaction rate $(v_{\rm rds,2})$ is given by:

$$v_{\rm rds,2} \equiv \lim_{c_{\rm ES,s} \to c_{\rm E}} k_2 c_{\rm ES,s} = k_2 c_{\rm E}.$$
 (24)

Therefore, the steady-state reaction rate of the entire reaction (v_s (= kc_E)) is given by substituting Eqs. 22 and 24 in Eq. 10 as:

$$\frac{1}{v_{\rm s}} = \frac{K_{\rm M}}{k_2 c_{\rm E} c_{\rm S}} + \frac{1}{k_2 c_{\rm E}} \left[= \frac{1}{k_2 c_{\rm E}} \left(1 + \frac{K_{\rm M}}{c_{\rm S}} \right) \right],\tag{25}$$

The reciprocal sum formula of Eq. 25 is rearranged as:

$$v_{\rm s} = \frac{k_2 c_{\rm E}}{1 + K_{\rm M}/c_{\rm S}}.$$
 (26)

Equation 26 is well-known Michaelis–Menten equation for steadystate enzyme kinetics⁴ and Eq. 25 indicates a linear relation between $1/v_s$ and $1/c_s$, which is often utilized for the kinetic analysis as is called Lineweaver–Burk plot.⁵

3.1.2 Competitive inhibition

Competitive inhibition, in which an inhibitor (I) is competitively bound to the free enzyme (E) to form an EI complex and inhibits the productive enzyme reaction, is one of inhibition modes in enzyme reactions. The reaction scheme is given by:

In this case, three hypothetical limiting cases are considered. Another reaction in addition to those of Eq. 21 concerns the EI complex formation and the corresponding limiting reaction rate $(v_{rds,3})$ is given by:

$$v_{\rm rds,3} \equiv \lim_{c_{\rm EL,s} \to c_{\rm E}} k_2 c_{\rm ES,s} = \lim_{c_{\rm EL,s} \to c_{\rm E}} k_2 \frac{c_{\rm ES,s}}{c_{\rm E,s}} \frac{c_{\rm E,s}}{c_{\rm EI,s}} c_{\rm EI,s}$$
$$= \lim_{c_{\rm EL,s} \to c_{\rm E}} k_2 c_{\rm EI,s} \frac{c_{\rm S}}{K_{\rm M}} \frac{K_{\rm EI}}{c_{\rm I}}$$
$$= k_2 c_{\rm E} \frac{c_{\rm S}}{K_{\rm M}} \frac{K_{\rm EI}}{c_{\rm I}}, \qquad (28)$$

where we define the competitive inhibition constant (K_{EI}) according to the nomenclature of the biochemistry field:

$$K_{\rm EI} \equiv \frac{c_{\rm E,s}c_{\rm I}}{c_{\rm EI,s}}.$$
(29)

The EI formation and its dissociation processes are, in this case, considered to be fast compared with the productive enzymatic reaction processes. Substituting Eqs. 22, 24, and 28 into Eq. 10 yields the steady-state kinetics equations for this case:

$$\frac{1}{v_{s}} = \frac{K_{M}}{k_{2}c_{E}c_{S}} + \frac{1}{k_{2}c_{E}} + \frac{K_{M}c_{I}}{k_{2}c_{E}c_{S}K_{EI}} \left\{ = \frac{1}{k_{2}c_{E}} \left[1 + \frac{K_{M}}{c_{S}} \left(1 + \frac{c_{I}}{K_{EI}} \right) \right] \right\}$$
(30)

$$v_{\rm s} = \frac{k_2 c_{\rm E}}{1 + \frac{K_{\rm M}}{c_{\rm S}} \left(1 + \frac{c_{\rm I}}{K_{\rm EI}}\right)}.$$
(31)

3.1.3 Uncompetitive inhibition

In this inhibition, an inhibitor (I) is bound to the ES complex to inhibit the productive reaction. The reaction scheme is given by:

The hypothetical limiting reaction rate ($v_{rds,4}$) of the case which concerns the ESI complex formation is given by:

$$v_{\text{rds},4} \equiv \lim_{c_{\text{ESI},s} \to c_{\text{E}}} k_2 c_{\text{ES},s} = \lim_{c_{\text{ESI},s} \to c_{\text{E}}} k_2 \frac{c_{\text{ES},s}}{c_{\text{ESI},s}} c_{\text{ESI},s}$$
$$= \lim_{c_{\text{ESI},s} \to c_{\text{E}}} k_2 c_{\text{ESI},s} \frac{K_{\text{ESI}}}{c_1} = k_2 c_{\text{E}} \frac{K_{\text{ESI}}}{c_1}, \qquad (33)$$

where we define the uncompetitive inhibition constant (K_{ESI}) according to the nomenclature of the biochemistry field:

$$K_{\rm ESI} \equiv \frac{c_{\rm ES,s}c_{\rm I}}{c_{\rm ESI,s}}.$$
(34)

Substituting Eqs. 22, 24, and 33 into Eq. 10 yields the steady-state kinetics equation for this case:

$$\frac{1}{v_{\rm s}} = \frac{K_{\rm M}}{k_2 c_{\rm E} c_{\rm S}} + \frac{1}{k_2 c_{\rm E}} + \frac{c_{\rm I}}{k_2 c_{\rm E} K_{\rm ESI}}$$
(35)

$$\begin{cases} = \frac{1}{k_2 c_{\rm E}} \left[1 + \frac{K_{\rm M}}{c_{\rm S}} + \frac{c_{\rm I}}{K_{\rm ESI}} \right] = \frac{\left(1 + \frac{c_{\rm I}}{K_{\rm ESI}} \right)}{k_2 c_{\rm E}} \left[1 + \frac{K_{\rm M}}{c_{\rm S} \left(1 + \frac{c_{\rm I}}{K_{\rm ESI}} \right)} \right] \right\},\\ v_{\rm s} = \frac{k_2 c_{\rm E} \left/ \left(1 + \frac{c_{\rm I}}{K_{\rm ESI}} \right)}{1 + \frac{K_{\rm M}}{c_{\rm S}} \left/ \left(1 + \frac{c_{\rm I}}{K_{\rm ESI}} \right)} \right]. \tag{36}$$

3.2 Ping-Pong Bi Bi mechanism

In this mechanism, the first substrate (A) binds to the enzyme (E) followed by its product (P) release and the generation of another form of the free enzyme (F). The second substrate (B) is bound to F followed by its product (Q) release and the regeneration of E:

$$E \xrightarrow{+A} EA \xrightarrow{k_2} -P F \xrightarrow{+B} FB \xrightarrow{k_4} -Q F.$$
 (37)

The system consists of 4 rdss, and the limiting reaction rates of the individual rdss are given as follows:

$$v_{\mathrm{rds},1} \equiv \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 c_{\mathrm{EA},s} = \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 \frac{c_{\mathrm{EA},s}}{c_{\mathrm{E},s}} c_{\mathrm{E},s}$$
$$= \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_2 c_{\mathrm{E},s} K_{\mathrm{s}1}$$
$$= k_2 c_{\mathrm{E}} K_{\mathrm{s}1} \quad \left(\text{with } K_{\mathrm{s},1} \equiv \frac{c_{\mathrm{EA},s}}{c_{\mathrm{E},s}} \right), \quad (38)$$

$$v_{\rm rds,2} \equiv \lim_{c_{\rm EA,s} \to c_{\rm E}} k_2 c_{\rm EA,s} = k_2 c_{\rm E},\tag{39}$$

$$v_{\mathrm{rds},3} \equiv \lim_{c_{\mathrm{F},s} \to c_{\mathrm{E}}} k_4 c_{\mathrm{FB},s} = \lim_{c_{\mathrm{F},s} \to c_{\mathrm{E}}} k_4 \frac{c_{\mathrm{FB}}}{c_{\mathrm{F},s}} c_{\mathrm{F},s}$$
$$= \lim_{c_{\mathrm{E},s} \to c_{\mathrm{E}}} k_4 c_{\mathrm{F},s} K_{s2}$$
$$= k_4 c_{\mathrm{E}} K_{s2} \quad \left(\text{with } K_{\mathrm{s},2} \equiv \frac{c_{\mathrm{FB},s}}{c_{\mathrm{F},s}} \right), \quad (40)$$
$$v_{\mathrm{rds},4} \equiv \lim_{c_{\mathrm{FB},s} \to c_{\mathrm{E}}} k_4 c_{\mathrm{FB},s} = k_4 c_{\mathrm{E}}. \quad (41)$$

Substitution of Eqs. 38–41 into Eq. 10 gives a reciprocal sum form of the steady-state enzyme kinetics:

$$\frac{1}{v_{s}} = \frac{1}{k_{2}c_{E}K_{s1}} + \frac{1}{k_{2}c_{E}} + \frac{1}{k_{4}c_{E}K_{s2}} + \frac{1}{k_{4}c_{E}}$$
$$= \frac{(k_{2} + k_{4})}{k_{2}k_{4}c_{E}} \left[1 + \frac{k_{4}}{K_{s,1}(k_{2} + k_{4})} + \frac{k_{2}}{K_{s,2}(k_{2} + k_{4})} \right]$$
$$= \frac{1}{k_{c}c_{E}} \left(1 + \frac{K_{M}^{A}}{c_{A}} + \frac{K_{M}^{B}}{c_{B}} \right), \tag{42}$$

where k_c , K_M^A , and K_M^B are the catalytic constant of the entire reactions and Michaelis constants for substrates A and B, respectively, according to the nomenclature of the biochemistry field, and are given by:

$$\frac{1}{k_{\rm c}} \equiv \frac{1}{k_2} + \frac{1}{k_4},\tag{43}$$

$$K_{\rm M}^{\rm A} \equiv \frac{k_4 c_{\rm A}}{K_{\rm s,1}(k_2 + k_4)},\tag{44}$$

$$K_{\rm M}^{\rm B} \equiv \frac{k_2 c_{\rm B}}{K_{\rm s,2}(k_2 + k_4)}.$$
(45)

Note here that Eq. 43 is also a reciprocal sum formula.

3.3 Ordered Bi Bi mechanism

In this mechanism, an enzyme (E) binds the first substrate (A) to form an EA complex, which furthermore binds the second substrate (B) to form an EAB complex. The EAB intermediate releases the products P and Q in turn to regenerate E:

$$\begin{array}{ccc} +A & +B \\ E \xrightarrow{k_1} & EA \xrightarrow{-B} EAB (\rightarrow EPQ) \xrightarrow{k_3} EQ & \xrightarrow{k_4} E. \\ \hline -A & -B & -P & -Q \end{array}$$

$$(46)$$

The system involves two sequential bi-directional processes, in which the forward process of the first bi-directional reaction can also be an rds. As a result, we have to consider the following 5 rdss, and the hypothetical limiting rates of the individual rdss are given as follows:

$$v_{\text{rds},1} \equiv \lim_{c_{\text{E},s} \to c_{\text{E}}} k_1 c_{\text{E},s} = k_1 c_{\text{E}}, \tag{47}$$
$$v_{\text{rds},2} \equiv \lim_{c_{\text{E},s} \to c_{\text{E}}} k_3 c_{\text{EAB},s} = \lim_{c_{\text{E},s} \to c_{\text{E}}} k_3 \frac{c_{\text{EAB},s}}{c_{\text{E},a,s}} \frac{c_{\text{EA},s}}{c_{\text{E},s}} c_{\text{E},s}$$

$$= \lim_{c_{E_s} \to c_E} k_3 c_{E_s} K_{s,1} K_{s,2}$$

= $k_3 c_E K_{s,1} K_{s,2}$. (48)

$$\left(\text{with } K_{s,1} \equiv \frac{c_{\text{EA},s}}{c_{\text{E},s}} \text{ and } K_{s,2} \equiv \frac{c_{\text{EAB},s}}{c_{\text{EA},s}}\right)$$
$$v_{\text{rds},3} \equiv \lim_{c_{\text{EA},s} \to c_{\text{E}}} k_{3}c_{\text{EAB},s} = \lim_{c_{\text{EA},s} \to c_{\text{E}}} k_{3}\frac{c_{\text{EAB},s}}{c_{\text{EA},s}}c_{\text{EA},s}$$
$$= \lim_{c_{\text{E},s} \to c_{\text{E}}} k_{3}c_{\text{E},s}K_{s,2} = k_{3}c_{\text{E}}K_{s,2}, \qquad (49)$$

$$v_{\rm rds,4} \equiv \lim_{c_{\rm EAB,s} \to c_{\rm E}} k_3 c_{\rm EAB,s} = k_3 c_{\rm E},\tag{50}$$

$$v_{\mathrm{rds},5} \equiv \lim_{c_{\mathrm{ED},s} \to c_{\mathrm{E}}} k_4 c_{\mathrm{EQ},s} = k_4 c_{\mathrm{E}}.$$
(51)

Substitution of Eqs. 47–51 in Eq. 10 gives the reciprocal form of the steady-state enzyme kinetics:

$$\frac{1}{v_{s}} = \frac{1}{k_{1}c_{E}} + \frac{1}{k_{3}c_{E}K_{s,1}K_{s,2}} + \frac{1}{k_{3}c_{E}K_{s,2}} + \frac{1}{k_{3}c_{E}} + \frac{1}{k_{4}c_{E}} \\
= \frac{(k_{3} + k_{4})}{k_{3}k_{4}c_{E}} \left[1 + \frac{k_{3}k_{4}}{k_{1}(k_{3} + k_{4})} + \frac{k_{4}}{K_{s,1}K_{s,2}(k_{3} + k_{4})} \right] \\
+ \frac{k_{4}}{K_{s,2}(k_{3} + k_{4})} \right] \\
= \frac{1}{k_{c}c_{E}} \left(1 + \frac{K_{M}^{A}}{c_{A}} + \frac{K_{M}^{B}}{c_{B}} + \frac{K_{1}^{A}K_{M}^{B}}{c_{A}c_{B}} \right),$$
(52)

where k_c , K_M^A , K_M^B , and K_I^A are the catalytic constant of the entire reactions, Michaelis constants for substrates A and B, and the inhibition constant for A, respectively, according to the nomenclature of the biochemistry field, and are given in this case by:

$$\frac{1}{k_{\rm c}} \equiv \frac{1}{k_3} + \frac{1}{k_4},\tag{53}$$

$$K_{\rm M}^{\rm A} \equiv \frac{k_3 k_4 c_{\rm A}}{k_1 (k_3 + k_4)},\tag{54}$$

$$K_{\rm M}^{\rm B} \equiv \frac{k_4 c_{\rm B}}{K_{\rm s,2}(k_3 + k_4)},\tag{55}$$

$$K_{\rm I}^{\rm A} \equiv \frac{c_{\rm A}}{K_{\rm s,1}},\tag{56}$$

The steady-state kinetic equations derived here are the typical ones in the biochemistry fields. For other complicated cases also, we can easily derive kinetic equations by defining the limiting rate equations for the individual rdss. One may add other rdss by considering additional intermediate in the modeling, but the corresponding equations will be simplified to those described here. As described in Introduction, the steady-state kinetic analysis is not powerful tool for characterization of the nature of intermediates, but it can be applied to easily analyze complicated systems.

4. Steady-State Voltammetry

4.1 Reversible system

Voltammetry at RDEs and UMEs often gives steady-state voltammograms. The equations in non-reciprocal forms describing steady-state current-potential curves look very complicated, and sometimes they are rewritten in the reciprocal sum forms.^{2,3} The current in electrochemical experiments is proportional to the net electron transfer reaction rate at the electrode, and the electrode reactions usually consist of several sequential reactions. Therefore, the steady-state current should be also expressed on the reciprocal sum concept. In this section, we will derive kinetic equations for some of the typical cases by using the reciprocal sum concept.

First of all, we will treat the reversible case. For simplicity, here, we may discuss the oxidation current in a solution containing a reductant (R) at a bulk concentration of $c_{\rm R}$. Diffusion-controlled limiting steady-state oxidation current ($i_{\rm d,ox}$) is given by:

$$R \rightleftharpoons O + ne^{-}$$
$$i_{d,ox} \equiv M_R c_R.$$
(57)

The proportional constant M_i depends on the electrochemical techniques used and is given as follows, for examples:²

$$M_i = 0.620 n FA D_i^{\frac{2}{3}} \omega^{\frac{1}{2}} v^{-\frac{1}{6}} = L_i \omega^{\frac{1}{2}}$$
(58)

(with $L_i \equiv 0.620 n FAD_i^{\frac{2}{3}} v^{-\frac{1}{6}}$) (at RDE),

$$M_i = \frac{n_i FAD_i}{r} = 2\pi n FD_i r \quad \text{(at semispherical UME)}, \quad (59)$$

$$M_i = \frac{4n_i F A D_i}{\pi r} = 4n F D_i r \quad \text{(at planar UME)}, \tag{60}$$

where n_i and D_i are the total number of the electrons and the diffusion coefficient of species *i* (*i* = 0 or R), respectively, *F* is Faraday constant, *A* is the surface area of the electrode, ω is the rotating speed in radian of RDE, ν is the kinetic viscosity of the solution, and *r* is the radius of UME.

When the electrode reaction is reversible, the surface concentration ratio of the redox couple (K_N) is given by Nernst equation:

$$K_{\rm N}\left(\equiv \frac{c_{\rm O,0}}{c_{\rm R,0}}\right) = \exp\left(\frac{nF(E-E^{\circ\prime})}{RT}\right),\tag{61}$$

where $c_{i,0}$ is the surface concentration of species *i*; *E* and $E^{\circ \prime}$ are the electrode potential and the formal potential, respectively; *R* and *T* are the gas constant and absolute temperature, respectively. The steady-state oxidation current ($i_{s,ox}$), in this case, is given by:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{i_{\rm d,ox}} + \frac{1}{i_{\rm rem,O}},$$
(62)

where $i_{\text{rem,O}}$ is the diffusion-limited current for the removal of the oxidant (O) (generated by the electrode reaction on the electrode surface) from the electrode surface and is called the removal-controlled current.³ Considering Eq. 61 and the situation that $c_{\text{R},0} = c_{\text{R}}$ under the limiting conditions, $i_{\text{rem,O}}$ is given by:

$$i_{\text{rem,O}} \equiv \lim_{c_{\text{R,O}} \to c_{\text{R}}} M_{\text{O}} c_{\text{O,O}} = M_{\text{O}} K_{\text{N}} c_{\text{R}}$$
$$= M_{\text{R}} \left(\frac{M_{\text{O}}}{M_{\text{R}}} \right) K_{\text{N}} c_{\text{R}} = M_{\text{R}} K_{\text{N}}^{\text{h}} c_{\text{R}}, \tag{63}$$

$$K_{\rm N}^{\rm h} \equiv \left(\frac{M_{\rm O}}{M_{\rm R}}\right) K_{\rm N} = \left(\frac{M_{\rm O}}{M_{\rm R}}\right) \exp\left(\frac{nF(E - E^{\circ\prime})}{RT}\right)$$
$$= \exp\left(\frac{\left[nF\left(E - E^{\circ\prime} + \frac{RT}{nF}\ln(M_{\rm O}/M_{\rm R})\right)\right]}{RT}\right)$$
$$= \exp\left(\frac{nF(E - E^{\rm h})}{RT}\right), \tag{64}$$

where E^{h} is the half-wave potential of the reversible wave (Nernstian half-wave potential) and is given as follows:

$$E^{\rm h} \equiv E^{\circ\prime} - \frac{RT}{nF} \ln(M_{\rm O}/M_{\rm R}) \left(= E^{\circ\prime} - \frac{2RT}{3nF} \ln\left(\frac{D_{\rm O}}{D_{\rm R}}\right) \text{ for RDE} \right).$$
(65)

Substituting Eqs. 57 and 63 into Eq. 62 leads to a reciprocal sum formula for the steady-state curve:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{M_{\rm R}K_{\rm N}^{\rm h}c_{\rm R}}.$$
(66)

Equation 66 is rewritten as:

$$i_{\rm s,ox} = \frac{M_{\rm R}c_{\rm R}}{1+1/K_{\rm N}^{\rm h}}.$$
 (67)

In the case of RDE, Eq. 67 is rewritten to express the steady-state current–potential curve for the reversible case:

$$i_{s,ox} = \frac{0.620nFA\omega^{\frac{1}{2}}\nu^{-\frac{1}{6}}D_{R}^{\frac{2}{3}}c_{R}}{1 + \exp\left(\frac{-nF(E - E^{h})}{RT}\right)}.$$
(68)

At $E \gg E^{\rm h}$, $i_{\rm rem,O}$ in Eq. 62 becomes infinity and $i_{\rm s}$ reaches the limiting value $(i_{\rm s,lim})$, which is identical with $i_{\rm d}$, in this case.

4.2 Quasi-reversible system

The steady-state oxidation current $(i_{s,ox})$, in this case, is composed of three hypothetical limiting rdss: the diffusion of R, the electrochemical oxidation of R, and the removal of O (as the backward electrochemical reaction and diffusion), and is given by:

$$\mathbf{R} \leftarrow \mathbf{O} + n\mathbf{e}$$
$$\frac{1}{i_{\text{s,ox}}} = \frac{1}{i_{\text{d,ox}}} + \frac{1}{i_{\text{e,ox}}} + \frac{1}{i_{\text{rem,O}}},$$
(69)

where $i_{e,ox}$ is the electrode kinetics-controlled limiting current (for the oxidation of R) and is given by:

$$i_{\rm e,ox} = nFAk_{\rm e,ox}c_{\rm R} \tag{70}$$

with

$$k_{\rm e,ox} = k^{\circ} \exp\left[\frac{(1-\alpha)F(E-E^{\circ'}{\rm rds})}{RT}\right]$$
(71)

where $k_{e,ox}$ and k° are the interfacial electron transfer rate constant (at *E*) and the standard one (at $E^{\circ'}_{rds}$), respectively. $E^{\circ'}_{rds}$ and α are the formal potential and the transfer coefficient of the rate-determining single electron transfer process. Substituting Eqs. 57, 63, and 70 into Eq. 69 yields the reciprocal sum formula for the steady-state current-potential curve in the quasi-reversible case:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{nFAk_{\rm e,ox}c_{\rm R}} + \frac{1}{M_{\rm R}K_{\rm N}^{\rm h}c_{\rm R}}.$$
(72)

At $E \gg E^{\circ'}_{rds}$ and $E \gg E^{h}$, $i_{e,ox}$ and $i_{rem,O}$ in Eq. 69 become infinity and $i_{s,ox}$ reaches the limiting value ($i_{s,ox,lim}$ (= $i_{d,ox}$ in this case)).

4.3 Irreversible system

When the electrode system is totally irreversible, the re-reduction

with

of the electrochemically generated O is ignored, and Eq. 69 is simplified as (see also Eq. 58):

$$R \to O + ne^{-}$$

$$\frac{1}{i_{\rm e,ox}} = \frac{1}{i_{\rm e,ox}} + \frac{1}{i_{\rm d,ox}} = \frac{1}{nFAk_{\rm e,ox}c_{\rm R}} + \frac{1}{L_{\rm R}\omega^{1/2}c_{\rm R}}.$$
(73)

According to Eq. 73, one may plot $1/i_{s,ox}(E)$ vs. $1/\omega^{1/2}$ to evaluate kinetic parameters (k° , α , and $E^{\circ'}_{rds}$) involved in $k_{e,ox}(E)$. The reciprocal sum formula in Eq. 73 is sometimes called Koutecký-Levich equation.^{2,3} However, the original paper of Koutecký & Levich⁶ deals with the limiting value of i_s ($i_{s,lim}$) for the electrocatalytic system in which the chemical reaction proceeds in solution. The limiting steady-state current $(i_{s,ox,lim})$ in the irreversible case becomes simply identical with $i_{d,ox}$ at $E \gg E^{\circ'}_{rds}$. Therefore, it is not desirable to refer to the expression of Eq. 73 as Koutecký-Levich equation. In addition, several authors tried to evaluate electrocatalytic performance of electrodes by using Eq. 73. However, Eq. 73 utilizes the concept of Butler-Volmer electrode kinetics⁷⁻⁹ that is based on the assumption of linear-free energy relationship (LFER) of a single electron transfer. LFER is essentially based on the assumption of non-specific reactions. Therefore, it would not be appropriate to evaluate electrocatalytic performance of electrodes from the kinetic parameters evaluated from $k_{e.ox}(E)$ obtained by the analysis using Eq. 73. Discussion on the n value from the $i_{d,ox}$ value evaluated from $1/i_{s,ox}(E)$ vs. $1/\omega^{1/2}$ is also useless. Electrocatalytic performance of electrodes should be evaluated based on another appropriate model(s) as described below.

4.4 Electrocatalytic system including a mono-directional electron transfer reaction

We may consider the following electrocatalytic system:

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$$\begin{array}{c} \text{R (in solution)} & \xrightarrow{\text{ansuson}} & \text{R (at electrode)} \\ & \xrightarrow{\text{electrocatalysis}} & \text{O (in solution).} \end{array}$$
(74)

Here we may also consider the following scheme for the electrocatalytic process. There exists the electrocatalytic site (C) for the oxidation of R on the electrode, which binds the reductant (R) to form a CR complex. The CR complex is electrochemically oxidized to CO to release the oxidant (O). The release process is assumed to be very fast compared with other processes.

$$C \xrightarrow{\text{catalysis}} CR \xrightarrow{\text{electron transfer}} CO(\xrightarrow{\text{fast}} C).$$

$$+R \xrightarrow{-ne^{-}} CO(\xrightarrow{-0} (75))$$

Therefore, the steady-state current $(i_{s,ox})$ -potential curve is given in a reciprocal sum formula by:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{i_{\rm d,ox}} + \frac{1}{i_{\rm ec,ox}} = \frac{1}{i_{\rm c,ox}} + \frac{1}{i_{\rm d,ox}} + \frac{1}{i_{\rm e,ox}},$$
(76)

where $i_{ec,ox}$ is the electrocatalysis-controlled limiting oxidation current, while $i_{c,ox}$ is the catalysis-controlled limiting oxidation current and is given by:

$$i_{\rm c,ox} = nFAk_{\rm c,ox}\Gamma_{\rm C},\tag{77}$$

where $k_{c,ox}$ is the catalytic constant and Γ_{C} is the total surface concentration of the catalyst. The parameter $k_{c,ox}\Gamma_{C}$ represents the catalytic activity of the electrode. $i_{e,ox}$ is electrode kinetics-controlled oxidation current and is, in this case, given by:

$$\dot{v}_{e,ox} = nFAk_{e,ox}\Gamma_{C},$$
(78)

with

$$k_{\rm e,ox} = k^{\circ} \exp\left[\frac{(1-\alpha)F(E-E^{\circ\prime}{}_{\rm rds})}{RT}\right],\tag{79}$$

where k° , α , and $E^{\circ'}{}_{rds}$ are for the CO/CR redox couple.

Considering Eqs. 77 and 78, $i_{ec,ox}$ is given as follow:

$$\frac{1}{i_{\text{ec,ox}}} = \frac{1}{nFAk_{\text{c,ox}}\Gamma_{\text{C}}} + \frac{1}{nFAk_{\text{e,ox}}\Gamma_{\text{C}}} = \frac{1}{nFAk_{\text{c,ox}}\Gamma_{\text{C}}}\left(1 + \frac{k_{\text{c,ox}}}{k_{\text{e,ox}}}\right).$$
(80)

Equation 80 means that electrocatalytic process is characterized by $k_{c,ox}\Gamma_C$ and $k_{c,ox}/k_{c,ox}$. However, to be more precise, since $k_{e,ox}$ depends on *E*, the system is characterized by 4 kinetics parameters: $k_{c,ox}\Gamma_C$, $k_{c,ox}/k^\circ$, α , and $E^{\circ'}_{rds}$. Since we are assuming the single electron process as an rds for the overall electron transfer process, α may be reasonably assumed to be 0.5. In order to evaluate the 4 (or 3) parameters, one has to analyze the overall steady-state current–potential curve. The analytical equation is given by substituting Eq. 80 into Eq. 76:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{i_{\rm d,ox}} + \frac{1}{i_{\rm ec,ox}} = \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{nFAk_{\rm c,ox}\Gamma_{\rm C}} \left(1 + \frac{k_{\rm c,ox}}{k_{\rm e,ox}}\right).$$
 (81)

Although one parameter concerning M_R is added for the expression of the overall steady-state current–potential curve, the M_R value may be evaluated (or calculated) by other experiments.

At $E \gg E^{\circ'}_{rds}$, $i_{e,ox}$ in Eq. 76 becomes infinity and $i_{s,ox}$ reaches the limiting value $(i_{s,ox,lim})$. However, the $i_{s,ox,lim}$, in this case, is not necessarily identical with $i_{d,ox}$, but is sometimes smaller than $i_{d,ox}$ as given by:

$$\frac{1}{i_{s,ox,lim}} = \frac{1}{i_{c,ox}} + \frac{1}{i_{d,ox}} \left(\text{or } i_{s,ox,lim} = \frac{i_{c,ox}i_{d,ox}}{i_{c,ox} + i_{d,ox}} \right).$$
(82)

Only in systems (or at electrodes) having very strong catalytic activity $(i_{c,ox} \gg i_{d,ox})$, $i_{s,ox,lim}$ becomes identical to $i_{d,ox}$. Therefore, simple evaluation of the *n* value from $i_{s,ox,lim}$ is very error prone.

Using the proportional constant L_R for RDE in Eq. 58, Eq. 82 is rewritten as:

$$\frac{1}{i_{\rm s,ox,lim}} = \frac{1}{L_{\rm R}\omega^{1/2}c_{\rm R}} + \frac{1}{i_{\rm c,ox}}.$$
(83)

According to Eq. 83, one may plot $1/i_{s,ox,lim}$ vs. $1/\omega^{1/2}$ to evaluate kinetic parameters concerning the catalytic process $(k_{c,ox}\Gamma_C)$. This analysis is exactly Koutecký–Levich-type plot and is only applicable to $i_{s,ox,lim}$ (not generally to $i_{s,ox}$).

4.5 Electrocatalytic system including a bi-directional electron transfer reaction

At the end, we would like to discuss another electrocatalytic system in which the interfacial electron-transfer process is bidirectional as shown below:

$$C \xrightarrow{k_{c,ox}} CR \xrightarrow{k_{e,ox}} CR \xrightarrow{electron transfer} CO. (84)$$

In this scheme, $k_{e,ox}$ is identical with $k_{e,ox}$ in Eq. 79, and $k_{e,red}$ is given by:

$$k_{\rm e,red} = k^{\circ} \exp\left[\frac{-\alpha F(E - E^{\circ'}_{\rm rds})}{RT}\right].$$
(85)

The steady-state concentration quotient of the redox CO/CR couple $(K_{s,C})$ is defined as follows:

$$K_{\rm s,C} \equiv \frac{\Gamma_{\rm CO,s}}{\Gamma_{\rm CR,s}}.$$
(86)

where $\Gamma_{i,s}$ is the steady-state surface concentration of the catalyst *i* (*i* = CO or CR). Considering that the steady-state reaction rate in the sequential reactions is identical with that of a mono-directional process, we can say that $i_{s,ox} = nFAk_{c,ox}\Gamma_{CO,s}$. Therefore, the electrocatalysis-controlled hypothetical limiting current involving a bi-directional electrode reaction is given by:

$$\frac{nFA}{i_{ec,ox}} = \lim_{\Gamma_{cos} \to \Gamma_C} \frac{1}{k_{c,ox} \Gamma_{CO,s}} + \lim_{\Gamma_{CRs} \to \Gamma_C} \frac{1}{k_{c,ox} \Gamma_{CO,s}}$$
$$= \frac{1}{k_{c,ox} \Gamma_C} + \lim_{\Gamma_{CRs} \to \Gamma_C} \frac{1}{k_{c,ox} K_{s,C} \Gamma_{CR,s}}$$
$$= \frac{1}{k_{c,ox} \Gamma_C} + \frac{1}{k_{c,ox} K_{s,C} \Gamma_C} = \frac{1}{k_{c,ox} \Gamma_C} \left(1 + \frac{1}{K_{s,C}}\right). \quad (87)$$

The overall steady-state current-potential curve is given by:

$$\frac{1}{i_{\rm s,ox}} = \frac{1}{i_{\rm d,ox}} + \frac{1}{i_{\rm ec,ox}} = \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{nFAk_{\rm c,ox}\Gamma_{\rm C}} \left(1 + \frac{1}{K_{\rm s,C}}\right).$$
 (88)

 $K_{\rm s,C}$ is a function of *E*. At $E \gg E^{\circ'}_{\rm rds}$, $K_{\rm s,C}$ becomes infinity, and $i_{\rm ec,ox} \cong nFAk_{\rm c,ox}\Gamma_{\rm C} = i_{\rm c,ox}$. Under the conditions, the $i_{\rm s,ox}$ value reaches the limiting value $(i_{\rm s,ox,lim})$, which is not necessarily identical with $i_{\rm d,ox}$, as shown by Eq. 82. Koutecký–Levich-type plot $(1/i_{\rm s,ox,lim} \text{ vs. } 1/\omega^{1/2})$ is applicable to $i_{\rm s,ox,lim}$, as given by Eq. 83.

 $K_{\rm s,C}$ may be given as $K_{\rm s,C} = k_{\rm e,ox}/(k_{\rm e,red} + k_{\rm c,ox})$ by using the steady-state approximation for $\Gamma_{\rm CR,s}$ ($k_{\rm e,ox}\Gamma_{\rm CR,s} = (k_{\rm e,red} + k_{\rm c,ox})\Gamma_{\rm CO,s}$). Considering this situation, Eq. 88 can be rewritten as:

$$\frac{1}{i_{s,ox}} = \frac{1}{i_{d,ox}} + \frac{1}{i_{ec,ox}} = \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{nFAk_{c,ox}\Gamma_{\rm C}} \left(1 + \frac{1}{K_{s,\rm C}}\right)$$
$$= \frac{1}{M_{\rm R}c_{\rm R}} + \frac{1}{nFAk_{c,ox}\Gamma_{\rm C}} \left(1 + \frac{1}{K_{\rm N,\rm C}} + \frac{k_{c,ox}}{k_{e,ox}}\right),$$
(89)

where $K_{N,C}$ is the Nernst equation for the single electron transfer rds of the catalysis and is given by:

$$K_{\rm N,C} \equiv \frac{k_{\rm e,ox}}{k_{\rm e,red}} = \exp\left[\frac{F(E - E^{\circ\prime}_{\rm rds})}{RT}\right].$$
(90)

Equations 89 and 90 indicate that the entire steady-state current– potential curve can be characterized by 4 parameters of the electrocatalytic process: $k_{c,ox}\Gamma_C$, $k_{c,ox}/k^\circ$, α , and $E^{\circ'}_{rds}$ by considering that the diffusion-limited parameter M_R may be evaluated by other methods.

4.6 For the reduction systems

Up to this point, we have been discussing the oxidation of R. In the case of the reduction of O, some modifications are required. Since the derivation is close similar to the case of the oxidation of R, the final equations are summarized here.

For the reversible case, Eqs. 57, 63, and 66, are, respectively, modified as follows:

$$\dot{v}_{\rm d,red} = -M_{\rm O}c_{\rm O},\tag{91}$$

$$i_{\rm rem,R} = -M_{\rm O}c_{\rm O}/K_{\rm N}^{\rm h},\tag{92}$$

$$\frac{1}{i_{\rm s,red}} = -\frac{1}{M_{\rm O}c_{\rm O}} - \frac{K_{\rm N}^{\rm h}}{M_{\rm O}c_{\rm O}}.$$
(93)

For the quasi-reversible case, Eqs. 70, 71, and 72 are, respectively, modified as follows:

$$i_{\rm e,red} = -nFAk_{\rm e,red}c_{\rm O},\tag{94}$$

$$k_{\rm e,red} = k^{\circ} \exp\left[\frac{-\alpha F(E - E^{\circ'} rds)}{RT}\right],\tag{95}$$

$$\frac{1}{i_{\rm s,red}} = -\frac{1}{nFAk_{\rm e,red}c_{\rm O}} - \frac{1}{M_{\rm O}c_{\rm O}} - \frac{K_{\rm N}^{\rm h}}{M_{\rm O}c_{\rm O}}.$$
(96)

For the irreversible system, Eq. 73 is modified as:

$$\frac{1}{i_{\rm s,red}} = \frac{1}{i_{\rm e,red}} + \frac{1}{i_{\rm d,red}} = -\frac{1}{nFAk_{\rm e,red}c_{\rm O}} - \frac{1}{L_{\rm O}\omega^{1/2}c_{\rm O}} \,. \tag{97}$$

For the electrocatalytic system including a mono-directional electron transfer reaction, Eqs. 77, 78, 79, and 81 are, respectively, modified as follows:

$$i_{\rm c,red} = -nFAk_{\rm c,red}\Gamma_{\rm C},\tag{98}$$

$$i_{\rm e,red} = -nFAk_{\rm e,red}\Gamma_{\rm C},\tag{99}$$

$$k_{\text{e,red}} = k^{\circ} \exp\left[\frac{-\alpha F(E - E^{\circ'} r_{\text{ds}})}{RT}\right], \qquad (100)$$

$$\frac{1}{i_{\text{s,red}}} = \frac{1}{i_{\text{d,red}}} + \frac{1}{i_{\text{ec,red}}} = -\frac{1}{M_{\text{O}}c_{\text{O}}} - \frac{1}{nFAk_{\text{c,red}}\Gamma_{\text{C}}}\left(1 + \frac{k_{\text{c,red}}}{k_{\text{e,red}}}\right). \qquad (101)$$

For the electrocatalytic system including a bi-directional electron transfer reaction, Eq. 89 is modified as:

$$\frac{1}{i_{s,red}} = \frac{1}{i_{d,red}} + \frac{1}{i_{ec,red}}$$
$$= -\frac{1}{M_{O}c_{O}} - \frac{1}{nFAk_{c,red}\Gamma_{C}}(1 + K_{s,C})$$
$$= -\frac{1}{M_{O}c_{O}} - \frac{1}{nFAk_{c,red}\Gamma_{C}}\left(1 + K_{N,C} + \frac{k_{c,red}}{k_{e,red}}\right). \quad (102)$$

5. Conclusions

We have confirmed that the reciprocal of the steady-state reaction rate in sequential reactions can be expressed as the sum of the reciprocals of the reaction rates of the individual rdss. This reciprocal sum formula is very useful in considering reaction kinetics. Each term of the summation corresponds to the hypothetical rds characterizing a part of the entire reaction. In the reciprocal sum concept, it is very important and useful to express the kinetics of each rds as a pseudo-first-order reaction. In each rds, the concentration of the corresponding intermediate is assumed to be identical to the initial concentration of the reactant to get the hypothetical limiting value of the reaction rate of the rds. The steady-state analysis is valuable in the situation that it provides a detailed picture of the properties of the catalyst in terms of kinetic parameters.

In the case of enzyme kinetics, this concept allows us to obtain an equation for the steady-state reaction rate without the need to calculate any complicated equations for intermediates. In addition, it gives clearer pictures of the rdss. When the reciprocal sum concept is applied to steady-state voltammetry, steady-state current-potential curves may be easily described without differential equations. Although the steady-state current can be measured with high precision, the kinetic parameters evaluated by steady-state kinetic analysis depend strongly on the mechanism to be considered. For example, oxygen reduction reaction (ORR) is frequently analyzed for the steady-state current on the irreversible reaction mechanism described in this study. However, almost all ORRs are catalyzed by the electrode materials used, and then ORR should be analyzed on any of electrocatalytic mechanisms. Such steady-state currents are often observed in catalytic electrode reactions, especially in enzymecatalyzed electrode reactions (bioelectrocatalytic reactions) and the steady-current is often given in the reciprocal sum form, as shown for example in Ref. 10. However, such reciprocal sum expressions are often deviated by inversion of the both side of complicated nonreciprocal expression that is deviated based on the steady-state assumption. We may propose to apply the present reciprocal sum concept to easily deviate wide varieties of steady-state currents including bioelectrocatalysis in the near future.

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Conflict of Interest

The authors declare no conflict of interest in the manuscript.

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