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'STEADY/EQUILIBRIUM APPROXIMATION' IN RELAXATION AND FLUCTUATION

I. PROCEDURE TO SIMPLIFY FIRST-ORDER REACTION

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A general procedure to simplify a complex first-order reaction by two approximations, the principle of fast equilibration and the steady-state approximation, is presented. Rate constants are classified into two groups: those of the order of unity and those of the order of $\varepsilon(\ll 1)$ or less, and are represented in the schemes by thick and thin arrows, respectively. The fast and the slow components are defined: from the fast component at least one thick arrow originates and from the slow component no thick arrow originates. Fast components are divided into several groups. In a group, the fast components are connected by thick arrows in both directions in each reaction step. When at least one thick arrow originates from the components in a group G and terminates on a component not belonging to group G (group G is open), then the steady-state approximation or principle of fast equilibration holds on each component in group G after an induction period T^0. When no thick arrow originating from group G is directed to components not belonging to group G (group G is closed), the principle of fast equilibration holds on the fast components in group G after T^0. The induction period T^0 is less than the order of $1/\varepsilon$.

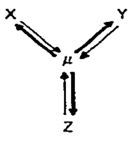
1. Introduction

Analysis of chemical relaxation and fluctuation is very useful for the investigation of molecular reaction mechanisms and characterization of their elementary steps in the fields of biochemistry, physiology and others (for reviews see refs. 2–5).

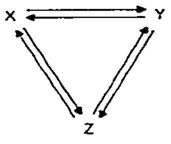
However, if the system is complex and involves many chemical components, proper analysis of experimental data of relaxation or fluctuation is very difficult. Theoretically, when a system contains n independent components, it is described in the neighborhood of equilibrium by $n-1$ different relaxation times. Experimentally, it is most common to observe only one or a few relaxation times. This suggests that many reactions proceed according to approximately simple reaction schemes. It is thus desirable to simplify a complex reaction scheme by suitable approximations. Thus far two simplifying procedures have been used [6]. One is the procedure to separate fast reaction steps from slow rate-determining steps (here we denote this by the principle of fast equilibration). If the exchange rates of certain reaction steps are large compared to those of other steps, these steps attain a quasi equilibrium and the slow (rate-determining) reaction steps can be described by assuming an equilibrium distribution of the components involved in fast reaction steps. (We show here that quasi equilibrium can sometimes be established rapidly even on the reaction steps whose exchange rate is not so large). Another procedure is known as the steady-state assumption or steady-state treatment in chemical reaction kinetics [6–9]. This assumption has been used so far without proof. It is assumed in this procedure that in the chemical kinetics of consecutive reactions, the concentration of each of the intermediates, which is very small during the reaction, is given by assuming that they are in a steady state, i.e., their time derivatives can be set to zero. However, it has been scarcely utilized for the analysis of relaxation and fluctuation in contrast with the principle of fast equilibration, because it has not been clear as to which cases this assumption can be applied. Although many attempts have been made on various reactions to determine the conditions under which the steady-state approximation is valid [10–14], this goal has not been achieved even in first-order reactions except for the simplest cases shown below. In the cases of higher order reactions, such a proof is more difficult [15]. In this work we generalize this treatment and denote it by the steady-state approximation.

Hammes and Alberty [17] examined the steady-state treatment in enzymatic consecutive reactions near equilibrium and proved that when only one intermediate exists between a substrate and a product, the smaller relaxation rate (the reciprocal of the relaxation time) among the two obtained exactly is approximately equal to the relaxation rate obtained by assuming a steady state of the intermediate, if the initial amount of substrate is much larger than the total amount of enzyme. Their proof also showed [6] that when the equilibrium concentration of an intermediate in a consecutive first-order reaction is much smaller than those of the reactant and product, the steady-state approximation for the intermediate is valid after a short induction period. Their proof, however, could not be extended to cases when more than two intermediates exist or when reactions are not consecutive.

Kijima and Gô [18] examined the tautomerization of sugars between pyranoses and furanoses in aqueous solution and demonstrated that the first-order branched interconversion between three components X, Y, Z through an intermediate μ (scheme I),



(I)



(II)

can be reduced to scheme II of the direct interconversion when the equilibrium content of μ is small enough compared with those of X, Y and Z. The proof clearly showed that the concentration of the intermediate μ rapidly attains a steady-state value in a characteristic time T which is much shorter than any of the relaxation times of scheme II. Thus, the steady-state approximation applied to the intermediate μ is always valid at $t \gg T$, irrespective of the initial value of μ.

In the present series of studies, we generalize the above work and present a unified theory on the two simplifying procedures (principles) and show under what conditions the two principles hold in first-order reactions.

In this paper, we first redefine the concepts of quasi equilibrium and quasi-steady state and also show what the two principles mean. Then, we present a general procedure to simplify first-order reactions by the above two principles without mathematical proof and also show some typical examples. The mathematical background of the procedure is presented in the subsequent paper [1] of this series.

We confine our discussion here to first-order reactions, which are most important and fundamental in the analysis of relaxation and fluctuation of biological systems. Receptor-agonist or protein-ligand interactions are regarded to be first-order reactions when the ligant is buffered (their concentrations are much larger than those of the proteins). In the chemical relaxation treatment originally introduced by Eigen [16], rate equations can often be linearized because the system usually deviates only slightly from equilibrium and a general theoretical treatment of rate equations is possible in a manner similar to that for first-order reactions. Applications of our theory to some of these cases will be given in a separate paper.

In this series of papers, we use the symbol O(1) and $O(\varepsilon')$ to denote a positive number of the order of unity and a positive number of the order of ε', respectively, or use them in place of the phrases 'order of unity' and 'order of ε', respectively. The expression $A \approx O(\varepsilon)$ means that the magnitude of A is of the order of ε and $A < O(1/\varepsilon)$ means that A is of O(1) or less, or that A is between O(1) and $O(1/\varepsilon)$, where $\varepsilon \ll 1$.

2. Procedure to apply the two simplifying principles

We treat a general first-order reaction scheme consisting of n components. The fundamental assumptions in this scheme are as follows.

(i) The rate constants are classified into two categories by their magnitude: those in one category are of O(1) by choosing an appropriate time scale (large rate constants) and those in the other are of the order of ε, ε^2, $\varepsilon^3, \ldots, \varepsilon^i$ (small rate constants), where $\varepsilon \ll 1$ and i is of O(1).

(ii) The rate constants can be zero. However, all the components are connected into a reaction scheme by the sequences of nonzero k_{ij} values, where k_{ij} represents the rate constant of conversion from component X_j to component X_i (the representation is reversed from that of Kijima and Gō [18]).

(iii) The reactions are reversible in every reaction step. When k_{ij} is not zero, k_{ji} is also not zero.

(iv) The number of components n is not so large that n and n^2 are of O(1).

(v) The system is not supplied with energy from other systems and thus the principle of detailed balance holds on each reaction step, i.e., $k_{ji}x_i(\infty) = k_{ij}x_j(\infty)$.

The rate constants of O(1) are represented by thick arrows in the reaction schemes and those of O(ε) or less are represented by thin arrows as shown in fig. 1. Using the above assumptions, the components are divided into two classes: the component from which only thin arrows originate belongs to one class and is denoted as the 'slow component'. The component from which at least one thick arrow originates belongs to another class and is denoted as the 'fast component' (fig. 1).

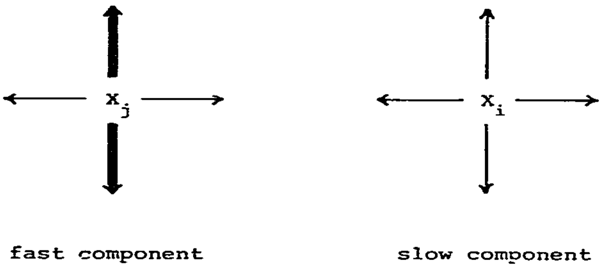


Fig. 1. The fast and slow components. A thick arrow represents a large rate constant of the order of unity and a thin arrow represents a small rate constant of the order of ε ($0 < \varepsilon \ll 1$) or less. From a fast component X_j, at least one thick arrow originates (there is at least one large rate constant k_{hj} ($h \neq j$)), and only thin arrows originate from a slow component X_i (any k_{hi} ($h \neq i$) is a small rate constant).

We further introduce the concept of the 'group' of fast components. The fast components belonging to a group are connected together in some way (consecutively or in a network, etc.) by thick arrows in both directions in each reaction step. The group is either open or closed. An open group is defined as a group of fast components from which at least one thick arrow originates and is directed toward the component not belonging to the group. On the other hand, a closed group is a group of fast components from which no thick arrow originates directed toward components not belonging to the group (the thick arrows are closed in the group). In the figure and examples shown below, the open group is surrounded by the dashed lines, while the closed group is surrounded by the continuous lines. An open group may sometimes consist of only one fast component, while a closed group contains more than two components. An example of an open and closed group is shown in fig. 2.

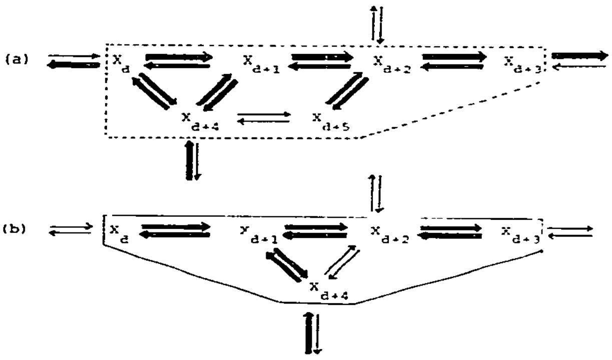


Fig. 2. Example of open and closed groups. (a) An open group of fast components, $X_d, \cdots, X_{d+5}$, connected by thick arrows in both directions. Thick arrows originate from components X_d and X_{d+3} in the group and terminate on components not belonging to the group. (b) A closed group of fast components, $X_d, \cdots, X_{d+4}$ connected by thick arrows in both directions. There are no thick arrows which originate from components in the group and terminate on components not belonging to the group.

The upper and lower groups of a group are defined as follows. If we can reach a group G starting from an open group F by tracing the sequence of thick arrows always in the positive direction of arrows, then open group F is the upper group of group G and group G is the lower group of open group F.

We assume that there are r slow components and m fast components ($r+m=n$). The slow components and their molar fractions are denoted by X_i and x_i, respectively ($i=1,\dots,r$), and the fast components and their molar fractions by X_j and x_j, respectively ($j=r+1,\dots,n$). Mass is conserved in the reaction and,

$$\sum_{i=1}^{n} x_i = 1. \tag{1}$$

The component whose concentration (molar fraction) is of O(1) is denoted as the major component and the component whose concentration is of O(ε) or less as the minor component.

The rate equations of the reaction are given as,

$$\frac{d}{dt}x_i = \sum_{j=1}^{n} k_{ij}x_j \quad (i=1,\dots\dots,n) \tag{2}$$

where,

$$k_{ii} = -\sum_{j=1(j\neq i)}^{n} k_{ji} \tag{3}$$

In the following text, the conclusions and procedures for simplifying the first-order reaction scheme are presented.

2.1. *'Steady/equilibrium approximation' on the fast components*

(i) For every fast component the following relation (eq. 4) holds after an appropriate induction period T° ($T^{\circ} < O(1/\varepsilon)$), as proved in the subsequent work [1],

$$\left|\frac{d}{dt}x_j\right| \lesssim O(\varepsilon)|k_{jj}x_j| \tag{4}$$

Eq. 4 shows that the net change of x_j is much smaller than the total outflux from it and hence than the influx to x_j. Eq. 4 is the most fundamental one obtained in our theory and is denoted as the 'steady/equilibrium relation'. This 'steady/equilibrium relation' is equivalent to (also proved in ref. 1),

$$|(x_j-\bar{x}_j)/x_j| \lesssim O(\varepsilon), \tag{5}$$

where $\bar{x}_j$ is the steady-state or local equilibrium value of x_j obtained by setting the rate equation of x_j equal to zero. And thus every fast component attains its quasi-steady-state or quasi-equilibrium value. Putting the time derivative of a fast component X_j equal to zero, i.e.,

$$\frac{d}{dt}x_j = \sum_{i=1}^{n} k_{ji}x_i = 0 \quad (j\text{: fast component}) \tag{6}$$

we can obtain x_j as a linear combination of other components to a good approximation. We denote eq. 6 as the 'steady/equilibrium equation' and the simplifying procedures making use of it as the 'steady/equilibrium approximation'.

(ii) Definition of some concepts: As stated above, the steady/equilibrium relation holds for a fast component X_j after T°.

When the relation,

$$|k_{jh}x_h - k_{hj}x_j| \lesssim O(\varepsilon)x_j \quad (k_{hj} \approx O(1)) \tag{7}$$

holds between X_j and neighboring component X_h in addition to the steady/equilibrium relation on X_j, we define X_j as being in quasi equilibrium with X_h.

Eq. 7 is equivalent to

$$x_j/x_h \doteqdot k_{jh}/k_{hj} \tag{8}$$

In this case, the net flow of mass per unit time from X_j to X_h is O(ε)x_j or less and vice versa.

When X_j is in quasi equilibrium with its neighboring component X_h and X_h is in quasi equilibrium with its neighboring component X_f, then X_j is in quasi equilibrium with X_f, even if X_f may not be the neighboring component of X_j. That is, when the ratio x_j/x_f is a constant equal to the ratio in true equilibrium at $t > T^{\circ}$, X_j and X_f are in quasi equilibrium.

When the relation

$$|k_{jh}x_h - k_{hj}x_j| > O(\varepsilon)x_j \tag{9}$$

holds between X_j and some neighboring components X_h, in addition to the steady/equilibrium relation on X_j, then, we define X_j as being in a quasi-steady state. The net flow of mass per unit time through X_j is larger than $O(\varepsilon)x_j$. When X_j is in a quasi-steady state at $t > T^o$, we define the steady-state approximation as holding (or can be applied) on X_j, even if X_j may also be in quasi equilibrium with some components.

When X_j is in quasi equilibrium with at least one neighboring component and not in a quasi-steady state, we simply state that X_j is in quasi equilibrium. When X_j is in quasi equilibrium after an appropriate induction period T^o, we define the principle of fast equilibration as holding (or can be applied) on X_j.

The expression that the steady/equilibrium approximation holds on X_j means that either the steady-state approximation or the principle of fast equilibration holds on X_j.

(iii) When the mass of O(1) exists in a group G or in its upper groups at $t = 0$, the approximate description (neglecting the terms of $O(\varepsilon)$ or less) of the rapid change of the fast components in group G before attaining a quasi-steady state or quasi equilibrium is given as follows. We eliminate components belonging neither to group G nor to its upper groups from the reaction scheme but leave all the thick arrows including those originating from group G or its upper groups and directed toward the eliminated components. And we obtain a reaction scheme consisting of only fast components belonging to group G and its upper groups. By solving the rate equations (eq. 2) for them, the components in group G are described by the sum of exponential terms with the relaxation times of O(1).

2.2. Open group

(i) At $t > T^o$, the fast components in an open group G attain a quasi-steady state or quasi equilibrium. The molar fractions of the components in group G are all of the same order of magnitude and are of $O(\varepsilon)$ or less.

(ii) The fast components in the open group on which the steady/equilibrium relation holds can be eliminated from the reaction scheme by applying the steady/equilibrium approximation, in general leaving the pathway through them, whether they are in a quasi-steady state or in quasi equilibrium. Because they are minor components, the mass conservation law (eq. 1) approximately holds on the reduced reaction scheme after elimination. The apparent rate constants of the reduced reaction scheme through the eliminated fast components belonging to an open group G are obtained as follows. Steady/equilibrium equations (eq. 6) for all X_j in group G are solved with respect to all x_j in group G. The obtained x_j expressed by linear combination of the components not belonging to group G are substituted into the remaining rate equations of eq. 2 and the apparent rate constants of the reduced scheme are obtained (cf. section 2.3).

(iii) The induction period T^o required for the establishment of a steady/equilibrium relation on an open group depends on the initial distribution of mass. But T^o is always less than $O(1/\varepsilon)$, i.e., the fast components in open groups are either in a quasi-steady state or in quasi equilibrium with some neighboring components or in both at $t \gtrsim O(1/\varepsilon)$. For example, when the initial concentration of at least one of the components in the following (a or b) is of O(1), T^o for an open group G is of O(1) or less: (a) the components belonging to group G or to its upper groups, or (b) the neighboring slow components of those above, from which thin arrows of $O(\varepsilon)$ are directed to them. On the other hand, if the initial concentrations of the components in the above (a and b) are all of $O(\varepsilon)$ or less, T^o for group G may sometimes be larger than of O(1) and nearly of $O(1/\varepsilon)$. The induction period T^o of each open group can thus be different (for details, cf. Appendix II in ref. 1 and section 2.3).

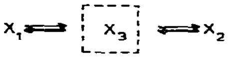
(iv) When the initial concentrations of fast components in an open group G are of O(1), T^o for group G is of O(1) and almost all the mass initially distributed in group G is transferred to the following components (c and d) in a definite manner determined by the equation given in Appendix III of ref. 1: (c) slow components to which thick arrows are directed from group G or from its lower groups; and (d) components in the lower closed groups.

(v) The apparent rate constants of the reduced scheme are of $O(\varepsilon)$ or less. If we redefine the fast component in the reduced scheme as the component from which at least one rate constant of O(ε) originates, we can apply again the two simplifying procedures to the new fast components.

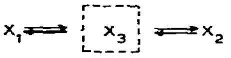
2.3. Examples of open groups

?.3.1. Simple schemes containing an open group

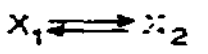
The following schemes (III-1 and III-2) are reduced to scheme IV by applying the steady/equilibrium approximation to the fast component, X_3, at $t \gtrsim T^\circ (T^\circ \lesssim O(1))$



(III-1)



(III-2)



(IV)

In scheme III-1, X_3 is in a quasi-steady state after T°. While in scheme III-2, X_3 is in quasi equilibrium with X_1 when, for example, $x_1(0) = 1$ and $x_2(0) = x_3(0) = 0$ and is in a quasi-steady state when $x_2(0) = 1$ and $x_1(0) = x_3(0) = 0$. The rate equations of scheme III-1 or III-2 are given by,

$$\frac{d}{dt}x_1 = -k_{31}x_1 + k_{13}x_3$$

$$\frac{d}{dt}x_2 = -k_{32}x_2 + k_{23}x_3$$

$$\frac{d}{dt}x_3 = k_{31}x_1 + k_{32}x_2 - (k_{13} + k_{23})x_3 \tag{10}$$

The rapid change of the fast component X_3 before attaining a quasi-steady/equilibrium is described approximately by the relaxation rate (the reciprocal of the relaxation time) of O(1), $k_{13} + k_{23}$ (section 2.1 (iii)) when $x_3(0) \approx O(1)$. The quasi-steady/equilibrium value of x_3 is obtained by putting $dx_3/dt = 0$ as

$$x_3 = (k_{31}x_1 + k_{32}x_2)/(k_{13} + k_{23}) \tag{11}$$

By substituting the above into the rate equations of X_1 and X_2 in eq. 10, we obtain the rate equations of the reduced scheme IV and the apparent rate constants are given as,

$$k_{21} \doteqdot \frac{k_{23}k_{31}}{k_1 + k_{23}}, \quad \text{for (III-1)}$$

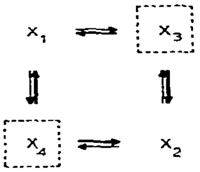
$$\doteqdot k_{23}k_{31}/k_{13}, \quad \text{for (III-2)}$$

$$k_{12} \doteqdot \frac{k_{13}k_{32}}{k_{13} + k_{23}}, \quad \text{for (III-1)}$$

$$\doteqdot k_{32}, \quad \text{for (III-2)}. \tag{12}$$

2.3.2. A looped reaction scheme

A looped reaction scheme (scheme V) with two intermediate fast components X_3 and X_4 is also reduced to scheme IV at $t > T^\circ (T^\circ \lesssim O(1))$ after application of the steady-state approximation to two groups consisting of X_3 and X_4, respectively,



(V)

The rapid change of X_3 and X_4 in attaining the quasi-steady state is described by the relaxation rates of O(1), $k_{13} + k_{23}$ and $k_{14} + k_{24}$, respectively.

Applying first the steady-state approximation separately to X_3 and X_4, scheme V is reduced to scheme VI:

$$\begin{array}{c} \\ \\ X_1 \\ \\ \\ \end{array} \begin{array}{c} k_{21}^{(3)} \\ \rightleftharpoons \\ k_{12}^{(3)} \\ k_{21}^{(4)} \\ \rightleftharpoons \\ k_{12}^{(4)} \end{array} \begin{array}{c} \\ \\ X_2 \\ \\ \\ \end{array} \tag{VI}$$

The apparent rate constants are

$$k_{21}^{(3)} = \frac{k_{23}k_{31}}{k_{13} + k_{23}}, \quad k_{12}^{(3)} = \frac{k_{13}k_{32}}{k_{13} + k_{23}}$$

$$k_{21}^{(4)} = \frac{k_{24}k_{41}}{k_{14} + k_{24}}, \quad k_{12}^{(4)} = \frac{k_{14}k_{42}}{k_{14} + k_{24}} \tag{13}$$

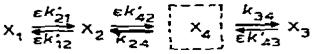
Scheme VI is equivalent to scheme IV, and the apparent rate constants of scheme IV are given as

$$k_{21} = k_{21}^{(3)} + k_{21}^{(4)}, \quad k_{12} = k_{12}^{(3)} + k_{12}^{(4)} \tag{14}$$

Scheme V is equivalent to the two-state model in the chemical excitation of a monomer at the postsynaptic membrane for a full agonist when the agonist concentration is constant [19–21] and is examined in detail elsewhere.

2.3.3. Simplest scheme in which the order of T^o is different depending on the initial condition

Let us imagine scheme VII,



(VII)

In the above scheme, we assume that k_{ij} and k'_{ij} values are of O(1). The fast component X_4 attains a quasi-steady state after the induction period T^o and the scheme is reduced to the next scheme (VIII),

$$X_1 \rightleftharpoons X_2 \rightleftharpoons X_3 \qquad \text{(VIII)}$$

However, T^o is different depending on the initial conditions.

(a) An example of initial conditions under which the steady-state approximation does not hold at $t \approx O(1)$.

We set the initial conditions as

$$x_1(0)=1, \quad x_2(0)=x_3(0)=x_4(0)=0. \qquad (15)$$

At $t \approx O(1)$,

$$x_2 \approx O(\varepsilon), \quad x_4 \approx O(\varepsilon^2), \qquad (16)$$

$$\frac{d}{dt} x_2 \doteqdot \varepsilon k'_{21} x_1 = \varepsilon k'_{21} \approx O(\varepsilon) \approx x_2 \quad \text{and}$$

$$\frac{d}{dt} x_4 \approx \varepsilon k'_{42} x_2 \approx O(\varepsilon^2) \approx x_4 \qquad (17)$$

Thus, the steady/equilibrium relation is not satisfied by X_4 and the steady-state approximation cannot be applied to X_4 at $t \approx O(1)$. When the time t approaches $O(1/\varepsilon)$, $x_2(t)$ becomes of the same order of magnitude as $x_1(t)$ and the steady-equilibrium relation holds and the steady-state approximation becomes valid for X_4.

(b) If at least one of $x_2(0)$, $x_3(0)$ and $x_4(0)$, is of O(1), then X_4 attains a quasi-steady state at $t \approx O(1)$ ($T^o \approx O(1)$).

The rapid change of x_4 before attaining the quasi-steady state is characterized by the relaxation rate of O(1), which is approximately equal to $k_{24}+k_{34}$. This rapid relaxation can hardly be observed in (a) because its relaxation amplitude is very small (of $O(\varepsilon^2)$).

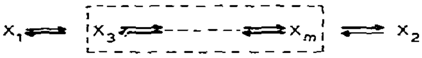
When $x_4(0) \approx O(1)$, almost all the mass on X_4 is transferred to X_2 and X_3 within the induction period and is retained on them for $T^o < t \approx O(1)$. The mass transferred to X_2 and X_3 from X_4 is given as (section 2.2 (iv)),

$$x_2 = \frac{k_{24}}{k_{24}+k_{34}} x_4(0), \quad x_3 = \frac{k_{34}}{k_{24}+k_{34}} x_4(0) \qquad (18)$$

The initial concentrations of X_2 and X_3 in the reduced scheme VIII are given by adding these transferred masses to $x_2(0)$ and $x_3(0)$, respectively. The initial concentration of X_1 in the reduced scheme is x_1 (0).

2.3.4. A general type of open group

If there is an open group of fast components between two components X_1 and X_2 and the group has at least one thick arrow terminating on X_1 or X_2 as exemplified by scheme IX or X,



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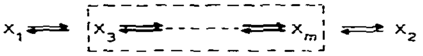
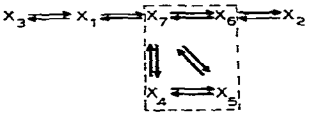


(X)

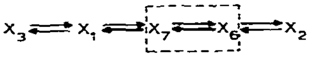
these schemes are simplified to scheme IV by applying the steady/equilibrium approximation to the fast components in the open group, however complicated the open group may be.

2.3.5. Fast components in an open group which attain quasi equilibrium and are simply eliminated in the reduced scheme for any initial conditions

If there is any extrusion of fast components in the open group which is connected to the other part of the reaction scheme via only one component, as shown in scheme XI, the components in the extruded part, X_4 and X_5, attain a quasi equilibrium with each other and with the component to which the extruded part is connected, X_7, when the steady/equilibrium relation holds on them, whatever the initial condition may be. This extruded part is completely neglected in the reduced reaction scheme and scheme XI is equivalent to scheme XII at $t > T^o$.



(XI)



(XII)

X_7 and X_6 are in a quasi-steady state and schemes XI and XII are reduced to scheme XIII by applying the steady-state approximation to X_7 and X_6.

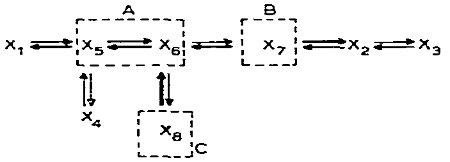
$$X_3 \rightleftharpoons X_1 \rightleftharpoons X_2 \qquad \text{(XIII)}$$

In scheme XI, when any one of $x_1(0)$, $x_2(0)$, $x_7(0)$, $x_4(0)$, $x_5(0)$ or $x_6(0)$ is of O(1), the induction period T° is of O(1) or less, while T° is nearly of $O(1/\varepsilon)$ ($T^\circ < O(1/\varepsilon)$) when $x_3(0) = 1$ and all others at $t = 0$ are of $O(\varepsilon)$ or less.

Approximately all the mass of O(1) initially located on X_4 X_5, X_6 and X_7 in scheme XI is transferred to X_1 and X_2 during the induction period and retained there for a time of O(1). Thus, the initial condition of the reduced scheme at T° is given as (section 2.2 (iv); also cf. Appendix III in ref. [1], eq. AIII-7),

$$\begin{aligned} x_1(T^\circ) &\doteqdot x_1(0) + A(x_4(0) + x_5(0) + x_7(0)) + Bx_6(0) \\ x_2(T^\circ) &\doteqdot x_2(0) + (1-A)(x_4(0) + x_5(0) + x_7(0)) \\ &\qquad + (1-B)x_6(0) \\ x_3(T^\circ) &\doteqdot x_3(0) \\ A &= \frac{k_{17}(k_{26} + k_{76})}{k_{17}(k_{26} + k_{76}) + k_{26}k_{67}} \\ B &= \frac{k_{17}k_{76}}{k_{26}(k_{17} + k_{67}) + k_{17}k_{76}} \end{aligned} \tag{19}$$

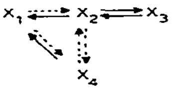
2.3.6. *A scheme (XIV) in which upper (or lower) groups exist*



(XIV)

In this scheme, thin arrows are assumed to be of $O(\varepsilon)$. The components X_5 and X_6, X_7 and X_8 form three different open groups A, B and C, respectively, and the groups B and C are the upper groups of group A.

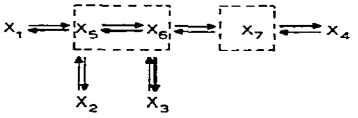
The induction period T° of group A is of O(1) when the initial concentration of at least one of the components except X_3 is of O(1) (cf. section 2.2 (iii)) while T° of group B (component X_7) is of O(1) when the initial concentration of at least one of the components X_2, X_5, X_6, X_7 and X_8 is of O(1). When $x_8(0) \approx O(1)$, the steady/equilibrium relation holds on X_8 at $t \approx O(1)$ and X_8 is in quasi equilibrium with X_6. At $t > T^\circ$, X_8 can be simply neglected. Moreover, when $x_1(0) = 1$ and the initial concentrations of the others are zero, the fast components X_5, X_6 and X_8 and the slow component X_1 are in quasi equilibrium with each other at $t \approx O(1)$, while X_7 is in a quasi-steady state. X_5, X_6, X_7 and X_8 attain a quasi-steady state or quasi equilibrium after induction period T^0 and scheme XIV reduces to scheme XV,



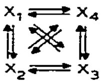
(XV)

where dotted arrows show the rate constants of $O(\varepsilon^2)$. Three slow components X_1, X_2 and X_4 connected with fast components belonging to group A and its upper group B come to react with each other in the reduced scheme. Group C (X_8) is simply neglected in the reduced scheme.

2.3.7. *A scheme in which four slow components connected with fast components belonging to two open groups reacting with each other come to interact with each other in a reduced scheme*



(XVI)



(XVII)

Scheme XVI reduces to scheme XVII after induction period T°.

2.4. *Closed group*

(i) On the fast components in a closed group G, the principle of fast equilibration holds at $t > T^\circ$ without exception. That is, they are all in quasi equilibrium with each other and their molar fractions are of O(1) or less.

(ii) After attaining a quasi equilibrium at $t > T^\circ$, the fast components in a closed group can be eliminated from the reaction scheme, leaving the sum of the components in the closed group as a slow component. At $t > T^{\circ}$, the rate equations for the fast components in a closed group can be approximately written as,

$$\frac{d}{dt} x_j = \sum_{j'(G)} k_{j,j'} x_{j'} \tag{20}$$

because the other terms can be neglected as they are small [1]. In eq. 20, the summation is on the components in group G.

For the fast components in open groups this relation does not hold. Thus, because eq. 4 holds at $t > T^{\circ}$, the following relation

$$\sum_{j'(G)} k_{jj'} x_{j'} = 0 \quad (j\text{:components in G}) \tag{21}$$

holds to a good approximation on closed groups. From these simultaneous equations, the proportion of each fast component x_j in group G to the sum of components in group G at quasi equilibrium is determined, which is the same as that at true equilibrium. The fast components in a closed group are in quasi equilibrium with each other in the group but not with the neighboring components, in contrast to those in the open group. Thus, they are not obtained as a linear combination of components not belonging to group G and the sum of components in group G must be left in the reduced scheme.

(iii) The apparent rate constants of the reduced reaction scheme, in which the components in a closed group G are eliminated leaving the sum of them as a slow component, are given as follows. These are the same as described by Colquhoun and Hawks [20].

(a) The apparent rate constants from component X_i not belonging to group G to the sum of the components of group G, $\Sigma_{j(G)} x_j$, is $\Sigma_{j(G)} k_{ji}$.

(b) The apparent rate constant from the sum of the components in group G, $\Sigma_{j(G)} x_j$, to component X_i not belonging to group G is $\Sigma_{j(G)} k_{ij} K_j$, where K_j is the proportion of x_j to $\Sigma_{j(G)} x_j$ at equilibrium.

(c) The apparent rate constants from the sum of the components in a closed group G, to the sum of the components in a closed group F, $\Sigma_{j'(F)} x_{j'}$, is given by using rule (a) and (b) as $\Sigma_{j'(F)} \Sigma_{j(G)} k_{j',j} K_j$.

(iv) The induction period T° before the establishment of quasi equilibrium depends on the initial conditions. However, T° is always less than $O(1/\varepsilon)$. For example, when the initial concentration of at least one component in the closed group G or in the upper groups of group G is $O(1)$, then T° for group G is of $O(1)$ or less. However, for example, when the initial concentration of all the components in closed group G and in its upper groups are of $O(\varepsilon)$ or less and that of at least one of the slow components, from which thin arrows of $O(\varepsilon)$ are directed to group G or to its upper groups, is of $O(1)$, then T° is nearly of $O(1/\varepsilon)$. Thus, the induction period of each closed group may be different.

2.5. The case in which both open and closed groups exist in a reaction scheme

(i) When the induction periods, T°, of the open and closed groups are different from each other, it is necessary to apply the steady/equilibrium approximation first to the groups which have reached quasi-equilibrium or quasi-steady state more rapidly than other groups. When either of the two principles holds on all groups in the reaction scheme after T°, it may be practically most convenient to apply first the principle of fast equilibration to the closed groups, to replace the groups by the sums of the components and to apply next the steady/equilibrium approximation to the open groups.

(ii) It is possible in some cases to apply further the two simplifying principles on the reduced reaction scheme by redefining the fast and slow components as stated in section 2.2 (v). By repeating the above procedures, we obtain finally the slowest overall reaction scheme.

2.6. Examples of closed groups and the reaction scheme in which both open and closed groups exist

2.6.1. The simplest example of a closed group Comparison of the rigorous solution with that obtained by applying the principle of fast equilibration

The next scheme (XVIII) reduces to scheme XIX after induction period T°, because the principle of fast equilibration holds on components X_2

and X_3 in the closed group;

$$X_1 \underset{\varepsilon k'_{12}}{\overset{\varepsilon k'_{21}}{\rightleftarrows}} \boxed{X_2 \underset{k_{23}}{\overset{k_{32}}{\rightleftarrows}} X_3} \quad \text{(XVIII)}$$

where k'_{21}, k'_{12}, k_{32} and k_{23} are assumed to be of O(1);

$$X_1 \underset{k_{1,(2+3)}}{\overset{k_{(2+3),1}}{\rightleftarrows}} (X_2+X_3) \quad \text{(XIX)}$$

The apparent rate constants in the reduced scheme are

$$k_{(2+3),1} = \varepsilon k'_{21}$$

$$k_{1,(2+3)} = \varepsilon k'_{12} k_{23}/(k_{23}+k_{32}) \quad (22)$$

The induction period T° depends on the initial condition. When either $x_2(0)$ or $x_3(0)$ is of O(1), then $T^{\circ} \lesssim O(1)$. On the other hand, if we set the initial condition as,

$$x_1(0) \doteqdot 1, \quad \text{and} \quad x_2(0) \approx x_3(0) \lesssim O(\varepsilon). \quad (23)$$

T° is near $O(1/\varepsilon)$, as shown in the following. Under the above condition and at $t \approx O(1)$, both dx_2/dt and x_2 (or dx_3/dt and x_3) are of $O(\varepsilon)$. So, the steady/equilibrium relation (eq. 4) does not hold on x_2 (or x_3) and the principle of fast equilibration cannot be applied to the closed group at $t \approx O(1)$.

The exact solutions of x_2 and x_3 at $t \gtrsim O(1)$ are given as follows (cf., for example, ref. 18) by neglecting the term of $e^{\lambda_3 t}$ (because $e^{\lambda_3 t}$ decreases rapidly at $t \approx O(1)$), where $-\lambda_3 \doteqdot k_{32} + k_{23}$ is the relaxation rate of O(1), whose approximate value is obtained from procedure (iii) in section 2.1.

$$x_2(t) = Be^{\lambda_2 t} + x_2(\infty)$$

$$x_3(t) = Ce^{\lambda_2 t} + x_3(\infty)$$

$$B = -x_2(\infty) + O(\varepsilon)$$

$$C = -x_3(\infty) + O(\varepsilon)$$

$$\lambda_2 \doteqdot \varepsilon k'_{21} + \frac{\varepsilon k'_{12} k_{23}}{k_{32} + k_{23}} \quad (24)$$

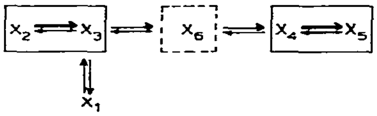
At $t \approx O(1)$, $e^{\lambda_2 t} \doteqdot 1 + \lambda_2 t$ and the two terms of O(1), $Be^{\lambda_2 t}$ and $x_2(\infty)$, in $x_2(t)$ are cancelled out, $x_2(t)$ becomes of $O(\varepsilon)$ or less and the term of $O(\varepsilon)$ in B cannot be neglected. Thus, the solution obtained by assuming fast equilibration between x_2 and x_3:

$$x_2(t) = -x_2(\infty) e^{\lambda'_2 t} + x_2(\infty), \quad \lambda'_2 = \lambda_2 \quad (25)$$

is not a good approximation of the exact solution, although λ_2 and $x_2(\infty)$ are obtained to a good approximation. When time t approaches $O(1/\varepsilon)$ and $x_2(t)$ reaches the order of $x_2(\infty)$, the above cancelling out disappears and eq. 25 becomes a good approximation of the exact solution (eq. 24). Thus, the principle of fast equilibration holds at $t > T^{\circ}(T^{\circ} < O(1/\varepsilon))$, whatever the initial condition is (cf. Appendix I in ref. 1).

2.6.2. A scheme containing both open and closed groups

Scheme XX is reduced to scheme XXI by applying the principle of fast equilibration and the steady-state approximation after an appropriate induction period $T^{\circ}(T^{\circ} < O(1/\varepsilon))$.



(XX)

$$X_1 \rightleftarrows (X_2+X_3) \rightleftarrows (X_4+X_5) \quad \text{(XXI)}$$

It is convenient practically to simplify in two steps. First, the principle of fast equilibration is applied to the closed groups and scheme XX is reduced to scheme XXII,

$$X_1 \rightleftarrows (X_2+X_3) \rightleftarrows \boxed{X_6} \rightleftarrows (X_4+X_5) \quad \text{(XXII)}$$

The apparent rate constants of scheme XXII are given as,

$$k_{(2+3),1} = k_{31}, \quad k_{1,(2+3)} = \frac{k_{13}k_{32}}{k_{23}+k_{32}}$$

$$k_{6,(2+3)} = \frac{k_{63}k_{32}}{k_{23}+k_{32}}, \quad k_{(2+3),6} = k_{36}$$

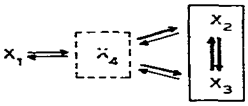
$$k_{(4+5),6} = k_{46}, \quad k_{6,(4+5)} = \frac{k_{64}k_{45}}{k_{45}+k_{54}} \quad (26)$$

Second, the steady/equilibrium approximation is applied to X_6 in the above scheme and we obtain scheme XXI. The apparent rate constants in the reduced scheme XXI are,

$$k_{(2+3),1} = k_{31}, \quad k_{1,(2+3)} = \frac{k_{13}k_{32}}{k_{23}+k_{32}}$$

$$k_{(4+5),(2+3)} = \frac{k_{46}k_{63}k_{32}}{k_{36}(k_{23}+k_{32})}, \quad k_{(2+3),(4+5)} = \frac{k_{64}k_{45}}{k_{45}+k_{54}} \quad (27)$$

2.6.3. *A branched and looped reaction scheme (XXIII), in which two principles of simplification can be applied*

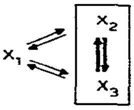


(XXIII)

When any one of $x_2(0)$, $x_3(0)$ or $x_4(0)$ is of O(1), the induction period T^o values of groups (X_2+X_3) and X_4 are both of O(1) or less and the above is reduced to the next scheme (XXIV).

$$X_1 \rightleftharpoons (X_2+X_3). \qquad \text{(XXIV)}$$

But when $x_1(0) \doteqdot 1$ and $x_2(0) \approx x_3(0) \approx x_4(0) \lesssim O(\varepsilon)$, X_4 attains a steady state at $t \approx O(1)$ whereas X_2 and X_3 attain a quasi equilibrium when t is nearly of $O(1/\varepsilon)$ $(T^o < O(1/\varepsilon))$. In the above case, scheme XXIII is reduced to scheme XXV at $t \approx O(1)$

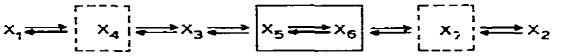


(XXV)

and is further reduced to scheme XXIV when t is nearly of $O(1/\varepsilon)$.

2.6.4. *An example in which a simplifying procedure is applied repeatedly*

In this and the next examples, the thick, thin and dotted arrows represent the rate constants of O(1), O(ε) and O(ε^2), respectively. The sequential reaction scheme XXVI is reduced to scheme XXVII by application of both simplifying procedures after a comparatively short induction period T^o $(T^o < O(1/\varepsilon))$.



(XXVI)

$$X_1 \rightleftharpoons X_3 \rightleftharpoons (X_5+X_6) \rightleftharpoons X_2 \qquad \text{(XXVII)}$$

In the reduced scheme XXVII, X_3 and (X_5+X_6) are newly defined as the fast components while X_1 and X_2 are regarded as the slow components. The steady-state approximation can be applied further to X_3 and (X_5+X_6). Scheme XXVII is reduced to scheme XXVIII after an appropriate induction period T^o $(T^o < O(1/\varepsilon^2))$

$$X_1 \rightleftharpoons X_2 \qquad \text{(XXVIII)}$$

In this scheme (XXVIII), all minor components at equilibrium, $X_3, \ldots, X_7$, which are all intermediates of the reaction between the major components X_1 and X_2, can be omitted finally by successive applications of the simplifying procedures. A more general treatment of components with a small equilibrium concentration will be given in a separate paper.

3. Discussions and conclusions

In the present work, we presented procedures to simplify complex first-order reactions by the steady/equilibrium approximation which is a generalization of two approximations known so far: the principle of fast equilibration and the steady-state approximation, without detailed mathematical proofs. The rigorous mathematical constructions of these procedures are given in the following paper [1]. We showed here that the time derivative of every fast component can be set to zero and the steady/equilibrium approximation holds on the fast components during most of the reaction time course after a comparatively short induction period T^o $(T^o < O(1/\varepsilon))$. Thus far, application of the principle of fast equilibration to the fast reaction step has been rather well studied and applied frequently to studies of the relaxation and fluctuation of chemical reactions together with the concept of the rate-determining step [20]. However, there has been no general study on the condition when the steady-state approximation holds even on the first-order reaction. In the present series of works, the unified treatment of both principles as the steady/equilibrium approximation is shown to be very useful.

We defined the open and closed groups of fast components. Components in closed groups C attain a quasi equilibrium and the proportion of each component to the sum of the components in group C is equal to that in true equilibrium. In the reduced scheme, only the sum of the components in group C is left as a slow component. On the other hand, components in open group G attain either a quasi-steady state or quasi equilibrium, depending on the reaction scheme and the initial conditions. When the fast components in group G are in a quasi-steady state or quasi equilibrium, they can be eliminated from the reaction scheme because their molar fractions are obtained as the linear combination of components not belonging to group G and are much smaller by a factor ε (or $\varepsilon^2, \varepsilon^3, \cdots, \varepsilon^i$) than the molar fraction of the largest components among the components neighboring group G or the upper groups of group G. But the pathway through them, in general, cannot be neglected with several exceptions.

The induction period T° of a group G before the two approximations are established is of O(1) or less, or near $O(1/\varepsilon)$ depending on the initial conditions. However, if we deal with only the changes of major components, we can show that to apply formally two approximations to every fast component at $t \approx O(1)$ and to eliminate them can be done without a large error. Such a treatment will be examined in a separate work together with the general treatment of minor components at equilibrium.

The criterion hitherto adopted widely for the applicability of the principle of fast equilibration to the fast reaction step is that the exchange rate, $k_{ij}x_j + k_{ji}x_i$, of the reaction step between X_j and X_i is large enough compared to those of other reaction steps. This criterion is sufficient but not always necessary because, for example, when the fast component in an open group is in quasi equilibrium with some neighboring components, the exchange rate is sometimes not so large (cf., section 2.3.5, scheme XI).

The other criterion for the fast equilibration, which has not been used correctly so far on the first-order reaction, is that the sum of rate constants $k_{ij} + k_{ji}$ in the reaction step between X_i and X_j is large enough. This is incorrect of course, because as shown in section 2.3.3, (scheme VII), both $k_{42} + k_{24}$ and $k_{43} + k_{34}$ are large enough compared to $k_{21} + k_{12}$, whereas X_4 is not in a quasi equilibrium but in a quasi-steady state.

The usefulness of the concept of the fast component is clearly demonstrated in the above discussions.

We assumed here that the kinetic constants of the reaction are classified into two categories; those of O(1) and those of $O(\varepsilon), O(\varepsilon^2), \cdots, O(\varepsilon^i)$ ($i \approx O(1)$) and that each reaction step is reversible. These two assumptions made the theory simple and clear. However, general features of a first-order reaction are not lost by these assumptions. An irreversible reaction can be treated as an extreme case of a reversible process in which the ratio of the kinetic constant of the backward reaction to that of the forward reaction is extremely small such as $O(\varepsilon^i):O(1)$.

We confine our discussion here only to a first-order reaction. In a higher order reaction, it has been impossible to determine the conditions under which the steady-state approximation holds, but a similar treatment to that in this work may be possible.

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