

The problem of distinguishability of rate constants in the two-step consecutive sequence

$A \rightarrow B \rightarrow C$

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Abstract: A detailed analysis of the absorbance versus time functional forms of the chemical species in the consecutive two-step unimolecular reaction scheme is presented. Distinguishability of rate constants is discussed with respect to the characterization of growth–decay and decay–growth curves and the rate constant – reaction step ambiguity. A new method based on the functional form of the absorption extremum involving the intermediate B with catalyst concentration is proposed to overcome the problem of rate constant assignments.

Key words: reaction mechanism, kinetics analysis, physical organic chemistry.

Résumé : On présente une analyse détaillée de l'absorption en fonction du temps des formes fonctionnelles des espèces chimiques dans deux étapes consécutives d'une réaction unimoléculaire. On discute de la différenciation des constantes de vitesse par rapport à la caractérisation des courbes de croissance–décroissance et décroissance–croissance et de la difficulté d'identifier l'étape déterminante de la constante de vitesse de la réaction. On propose une nouvelle méthode basée sur la forme fonctionnelle de l'extremum d'absorption impliquant l'intermédiaire B avec une concentration du catalyseur pour surmonter le problème d'attribution des constantes de vitesse.

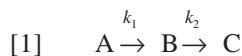
Mots clés : mécanisme réactionnel, analyse cinétique, chimie organique physique.

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Introduction

The determination of rate laws and reaction mechanisms is of fundamental importance in chemistry. Indeed, the discipline of physical organic chemistry was born with these concepts at its centre and continues to be rich in this tradition in current pure and applied organic chemistry research. Most notably, the area of kinetic and spectroscopic characterization of reaction intermediates has benefitted greatly from reaction mechanism analysis. There exist cornerstone references on the subject of solution-phase chemical kinetics which describe in detail various kinetic systems and the time-dependent behaviour of their component chemical species (1–6).

One such reaction scheme is the two-step consecutive unimolecular transformation given in eq. [1] with associated rate constants k_1 and k_2 . Note that the subscript labels for the rate constants, 1 and 2, refer to step 1 and step 2, respectively, in the sequence.



This particular kinetic system is well known with several examples cited in the literature (1–14). The time-dependent

absorbance functions of each of the chemical species, A, B, and C are given in eqs. [2a]–[2c] with initial conditions $A_A(0) = A_A^0$, $A_B(0) = 0$, and $A_C(0) = 0$ at time zero (1–6).

$$[2a] \quad A_A = A_A^0 \exp[-k_1 t]$$

$$[2b] \quad A_B = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) \{ \exp[-k_2 t] - \exp[-k_1 t] \}$$

$$[2c] \quad A_C = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left\{ 1 - \frac{1}{k_1 - k_2} (k_1 \exp[-k_2 t] - k_2 \exp[-k_1 t]) \right\}$$

where A_A^0 represents the initial absorbance of A at time zero, and ϵ_A , ϵ_B , and ϵ_C represent the absorption coefficients of A, B, and C, respectively.

This paper presents a new detailed analysis of this kinetic system encompassing that found in the traditional works cited above. More importantly, the less well-documented experimental problem of assigning calculated rate constants, k_1 and k_2 , obtained from nonlinear regression analysis to the appropriate steps in the reaction sequence is addressed. This problem has been documented by a few authors, and essentially two methods have been described which deal with the ambiguity of rate constant assignments (7–15). These methods rely either on inclusion of additional chemical knowledge or on analysis of the variation of absorption coefficients to remove the ambiguity. A method based only on the behaviour of the extremum absorption of the intermediate B is presented here. This method is universal and is particularly advantageous for cases where required additional

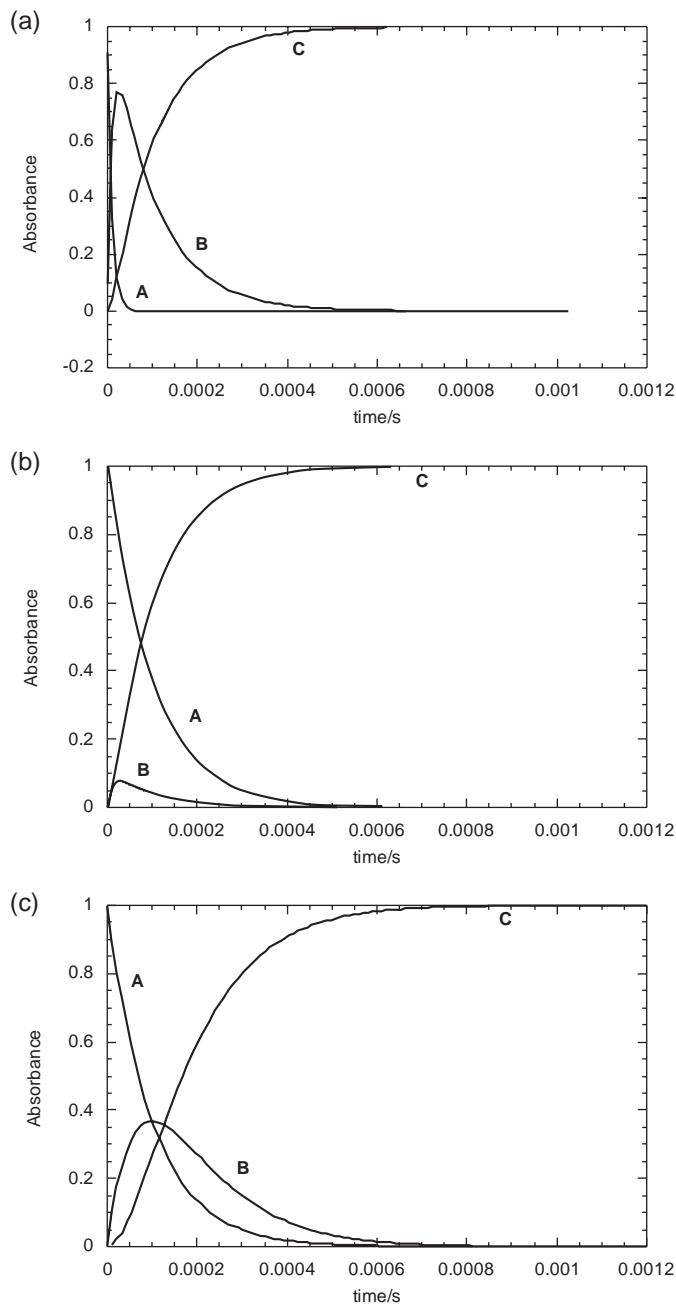
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This paper is dedicated to Jerry Kresge in recognition of his many achievements in chemistry.

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Fig. 1. Absorbance–time functions for species A, B, and C fitted according to eqs. [2a]–[2c]: (a) $k_1 = 10^5 \text{ s}^{-1}$, $k_2 = 10^4 \text{ s}^{-1}$, $A_A^0 = 1$, $\epsilon_B/\epsilon_A = 1$, $\epsilon_C/\epsilon_A = 1$; (b) $k_1 = 10^4 \text{ s}^{-1}$, $k_2 = 10^5 \text{ s}^{-1}$, $A_A^0 = 1$, $\epsilon_B/\epsilon_A = 1$, $\epsilon_C/\epsilon_A = 1$; and (c) fitted according to eqs. [5a]–[5c]: $k_1 = 10^4 \text{ s}^{-1}$, $k_2 = 10^4 \text{ s}^{-1}$, $A_A^0 = 1$, $\epsilon_B/\epsilon_A = 1$, $\epsilon_C/\epsilon_A = 1$.



experimental information to overcome the problem may be difficult to obtain.

Explanations given in the literature of the apparent mathematical ambiguity in this kinetic scheme vary depending on the author and are either incomplete or somewhat cloaked in confusing language. In addition to presenting a new method, it is intended to also provide a unified analysis of the problem which ties all ideas together in a concise and clear fashion without sacrificing rigour.

Properties of the two-step consecutive sequence

We begin by reviewing the properties of the absorbance–time functions pertaining to eq. [1]. It is instructive to examine the following three cases: (i) $k_1 > k_2$, (ii) $k_2 > k_1$, and (iii) $k_1 = k_2 = k$. For case (i), eqs. [2a]–[2c] apply as written. For case (ii), eqs. [3a]–[3c] given below apply.

$$[3a] \quad A_A = A_A^0 \exp[-k_1 t]$$

$$[3b] \quad A_B = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_2 - k_1} \right) \{ \exp[-k_1 t] - \exp[-k_2 t] \}$$

$$[3c] \quad A_C = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left\{ 1 - \frac{1}{k_2 - k_1} (k_2 \exp[-k_1 t] - k_1 \exp[-k_2 t]) \right\}$$

It should be noted that the sets of eqs. [2a]–[2c] and [3a]–[3c] are identical. Multiplication of the numerator and denominator by -1 in the terms $k_1/(k_1 - k_2)$ and $1/(k_1 - k_2)$ in eqs. [2b] and [2c], respectively, leads immediately to eqs. [3b] and [3c]. For case (iii), setting $k_1 = k_2 = k$ in eqs. [2a]–[2c] results in indeterminate forms for A_B and A_C . A mathematical manipulation employed to overcome this problem is to express one rate constant as a multiple of the other and then to find the limiting expressions as this factor approaches unity. Hence, setting $k_2 = nk_1$ where $0 < n < \infty$ leads to the set of eqs. [4a]–[4c].

$$[4a] \quad A_A = A_A^0 \exp[-k_1 t]$$

$$[4b] \quad A_B = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{1}{n-1} \right) \{ \exp[-k_1 t] - \exp[-nk_1 t] \}$$

$$[4c] \quad A_C = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left\{ 1 - \frac{1}{n-1} (n \exp[-k_1 t] - \exp[-nk_1 t]) \right\}$$

Taking the limit of eqs. [4a]–[4c] as $n \rightarrow 1$ and using l'Hôpital's rule (16) results in eqs. [5a]–[5c].

$$[5a] \quad A_A = A_A^0 \exp[-kt]$$

$$[5b] \quad A_B = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) kt \exp[-kt]$$

$$[5c] \quad A_C = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \{ 1 - (1 + kt) \exp[-kt] \}$$

As is readily apparent, the set of eqs. [4a]–[4c] conveniently describes all three cases: case (i) occurs when $0 < n < 1$, case (ii) occurs when $1 < n < \infty$, and case (iii) occurs when $n = 1$. This manipulation of representing one rate constant as a multiple of the other is very useful and will become important in later analyses.

A detailed analysis of the forms of the functions describing the time-dependent absorbances of species A, B, and C reveals a number of important characteristics. Figs. 1a–c illustrate graphically the three absorbance–time functions for each of the cases given above. The absorbance–time func-

Table 1. Possible chemical species observed at a given monitoring wavelength.

Number of chemical species observed at given monitoring wavelength	Number of possible experimental outcomes	Possible chemical species observed at monitoring wavelength
1	3	A or B or C
2	3	A and B, or A and C, or B and C
3	1	A, B, and C

tion for species A is simply a first-order decay with an initial absorbance equal to A_A^0 and a limiting final absorbance ($t \rightarrow \infty$) equal to zero. The absorbance–time function for species B is characterized as concave down with a single maximum. The initial and limiting final absorbances are both equal to zero. The maximum point in the absorbance–time function for B has a value given by

$$[6a] \quad A_B^{\max} = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{1}{n-1} \right) [n^{-1/(n-1)} - n^{-n/(n-1)}]$$

occurring at

$$[6b] \quad t_m = \frac{1}{k_1} \left(\frac{\ln n}{n-1} \right)$$

where $k_2 = nk_1$, $0 < n < \infty$. Note that the co-ordinates of the maximum for each of the cases given above are

$$t_m = \frac{1}{k_1 - k_2} \ln \left(\frac{k_1}{k_2} \right)$$

and

$$A_B^{\max} = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) \left[\left(\frac{k_1}{k_2} \right)^{-k_2/(k_1 - k_2)} - \left(\frac{k_1}{k_2} \right)^{-k_1/(k_1 - k_2)} \right]$$

when $k_1 > k_2$ ($0 < n < 1$) or $k_1 < k_2$ ($1 < n < \infty$), and $t_m = 1/k$ and $A_B^{\max} = A_A^0 (\epsilon_B/\epsilon_A) \exp(-1)$ when $k_1 = k_2$ ($n = 1$). It may be verified that the function in n in eq. [6a] approaches a limiting value of $\exp(-1) = 0.3679$ as n approaches 1 (see Appendix A). Finally, the absorbance–time function for species C is a sigmoidal growth and is characterized as having an inflection point dividing the curve into a concave up portion before the inflection point and a concave down portion after it. The value of t corresponding to the inflection point is commonly referred to as the induction period. From eq. [4c] this inflection point occurs exactly at the value of t corresponding to the maximum of the absorbance–time function of B (see eq. [6b]), and has a corresponding absorbance value given by

$$[7] \quad A_C^{\text{inf}} = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left\{ 1 - \frac{1}{n-1} (n^{(n-2)/(n-1)} - n^{-n/(n-1)}) \right\}$$

The initial and limiting final absorbances are equal to zero and $A_A^0 (\epsilon_C/\epsilon_A)$, respectively. The point (0, 0) corresponds to a minimum; whereas, the limiting absorbance of $A_A^0 (\epsilon_C/\epsilon_A)$ corresponds to a maximum. Note also that the absorbance curve for C is invariant to the transposition of values for k_1 and k_2 (see Fig. 1).

Possible experimental outcomes

If a chemical system is described by the reaction mechanism of eq. [1], then there are several experimental possibilities for observing the chemical species depending on the number of species observed at any one time according to a particular property. For example, in the case of a kinetics experiment conducted using UV-vis spectrophotometry, if each chemical species has a characteristic monitoring wavelength unique to that species, then it is possible to observe the absorbance–time evolution of each component separately. In practice such an ideal situation is rarely encountered, and hence usually several combinations of species may be observed at once. Table 1 summarizes the various possible experimental situations, and Tables 2–4 list the possible absorbance–time functions for observing one, two, and three chemical species at a given monitoring wavelength, respectively. Note that the entries in Table 2 correspond to the equations and properties given in the previous section.

At this point it is important to examine the general case of observing three chemical species (A, B, and C) with respect to isobestic points. There are three cases of isobestic points to consider: (i) $\epsilon_A = \epsilon_B$, (ii) $\epsilon_A = \epsilon_C$, and (iii) $\epsilon_B = \epsilon_C$. The expression in Table 4 is simplified under these conditions and the possible functional outcomes are greatly reduced as is shown by the entries in Table 5.

Properties of growth–decay and decay–growth curves

Suppose that a kinetics experiment is performed in which the appearance and disappearance of B is monitored directly as a function of time. Typically, the array of data points ($t_i, A_{B,i}$) is fitted by means of a nonlinear regression algorithm to a model given by

$$[8] \quad y = a[\exp(-bt) - \exp(-ct)]$$

where a , b , and c are parameters to be determined. Parameters b and c refer to the two rate constants and are thus always positively valued; whereas, parameter a is a pre-exponential factor that can be positively or negatively valued. Note that eq. [8] is the general form of eqs. [2b], [3b], and [4b]. It is worth examining in detail the functional behaviour of eq. [8] which will reveal information about the location of extrema (maxima, minima, and inflection points) and the directions of concavity of the curve between extrema.

Let us consider all parameters to be positively valued. We begin by taking first and second derivatives of y with respect to t

$$[9a] \quad \frac{dy}{dt} = a[-b \exp(-bt) + c \exp(-ct)]$$

Table 2. Absorbance–time functions for observing one chemical species in eq. [1].

Case	Absorbance–time function and boundary conditions	Function type
A	$A_A(t) = A_A^0 e^{-k_1 t}$ $A_A(0) = A_A^0$ $A_A(\infty) = 0$	Decay
B	$A_B(t) = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} - A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_1 t}$ $A_B(0) = 0$ $A_B(\infty) = 0$	Growth–decay if $k_1 > k_2$ or $k_2 > k_1$
C	$A_C(t) = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) - A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left(\frac{k_2}{k_1 - k_2} \right) e^{-k_1 t}$ $A_C(0) = 0$ $A_C(\infty) = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right)$	Sigmoidal growth if $k_1 > k_2$ or $k_2 > k_1$

$$[9b] \quad \frac{d^2 y}{dt^2} = a[b^2 \exp(-bt) - c^2 \exp(-ct)]$$

and find that an extremum occurs at

$$[10] \quad t = \frac{1}{c-b} \ln \left(\frac{c}{b} \right)$$

when $dy/dt = 0$. The value of y at this extremum is

$$y_m = a \left[\left(\frac{c}{b} \right)^{-b/(c-b)} - \left(\frac{c}{b} \right)^{-c/(c-b)} \right]$$

The identity of the extremum and the direction of concavity at this point are then found by substituting eq. [10] into the expression for the second derivative (eq. [9b]) and determining the sign of the value (16). Hence, eq. [8] exhibits a *minimum* (concave up) when $b > c$ and a *maximum* (concave down) when $c > b$. Figs. 2a and 2b illustrate the two possible curves: a positively valued growth–decay curve when $c > b$, and a negatively valued decay–growth curve when $b > c$. Note that if the pre-exponential factor a is negatively valued, the above analysis results only in the transposition of b and c in all equations; however, the conclusions of Theorems 1–3 (vide infra) remain unchanged. Table 6 summarizes the important conclusions of the above functional analysis on eq. [8].

From the above analysis we may then establish a simple inspection method to identify which rate constant corresponds to which part of the curve:

Theorem 1. Given a growth–decay or decay–growth function of the form of eq. [8] with positively valued parameters a , b , and c , the rate constant corresponding to the growth part of the curve is the one associated with a minus sign preceding the exponential factor containing that rate constant.

Furthermore, from Table 6 we can also draw the following conclusions regarding the correspondence between the rate constant for the growth and its relative magnitude.

Theorem 2. For a growth–decay function of the form of eq. [8] with positively valued parameters a , b , and c , the growth part of the curve corresponds to

the larger rate constant; whereas, the decay part of the curve corresponds to the smaller rate constant.

Theorem 3. For a decay–growth function of the form of eq. [8] with positively valued parameters a , b , and c , the growth part of the curve corresponds to the smaller rate constant; whereas, the decay part of the curve corresponds to the larger rate constant.

A similar analysis of the general expression in eq. [11] applicable to any

$$[11] \quad y = a \exp(-\alpha t) - b \exp(-\beta t) + c$$

of the possible experimental growth–decay and decay–growth curves listed in Tables 3 and 4 reveals the same functional properties as summarized in Table 7.

The problem of distinguishability of rate constants

Consider the growth–decay curves for the intermediate B shown in Figs. 1b and 1c. Both curves are fitted to the expression given in eq. [2b] where the values $k_1 = 10^5 \text{ s}^{-1}$ and $k_2 = 10^4 \text{ s}^{-1}$ are applicable to Fig. 1b, and the values $k_1 = 10^4 \text{ s}^{-1}$ and $k_2 = 10^5 \text{ s}^{-1}$ are applicable to Fig. 1c. The subscripts 1 and 2 in the rate constant descriptors strictly refer to steps 1 and 2, respectively, in the reaction sequence. Comparison of the forms of these two growth–decay curves indicates that they have identical functional behaviours, *except that their amplitudes differ*. More precisely, the similarities are that the growth part in each curve corresponds to the faster rate, 10^5 s^{-1} (see Theorem 2), the decay part of each curve corresponds to the slower rate, 10^4 s^{-1} (see Theorem 2), and the occurrence of the maximum in each case is at $t = 2.56 \times 10^{-5} \text{ s}$ (using eq. [6b]). The difference is that the amplitude of the maximum absorbance given by eq. [6a] is 0.77 for $k_2/k_1 = n = 0.1$ (Fig. 1b) and 0.077 for $k_2/k_1 = n = 10$ (Fig. 1c). From this example, it is evident that the faster rate constant associated with the growth in each curve corresponds to either the first or second steps in the reaction sequence. Likewise, the slower rate constant associated with the decay in each curve corresponds to either the first or second steps in the reaction sequence. The conclusion is that one cannot assign the two rate constants to their respective

Table 3. Absorbance–time functions for observing two chemical species in eq. [1].

Case	Absorbance–time function and boundary conditions	Function type
A and B	$A(t) = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t}$ $+ A_A^0 \left[1 - \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) \right] e^{-k_1 t}$ $A(0) = A_A^0$ $A(\infty) = 0$	<p>Sum of two decays if $\frac{\epsilon_B}{\epsilon_A} < \frac{k_1 - k_2}{k_1}$ and $k_1 > k_2$</p> <p>Growth–decay if $\frac{\epsilon_B}{\epsilon_A} > \frac{k_1 - k_2}{k_1}$ and $k_1 > k_2$</p> <p>N.B.: It is impossible to have a sum of two growths or a decay–growth curve.</p>
A and C	$A(t) = A_A^0 \left[1 + \left(\frac{\epsilon_C}{\epsilon_A} \right) \left(\frac{k_2}{k_1 - k_2} \right) \right] e^{-k_1 t}$ $- A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right)$ $A(0) = A_A^0$ $A(\infty) = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right)$	<p>Sum of two decays if $\frac{\epsilon_C}{\epsilon_A} < \frac{k_2 - k_1}{k_2}$ and $k_2 > k_1$</p> <p>Decay–growth if $\frac{\epsilon_C}{\epsilon_A} > \frac{k_2 - k_1}{k_2}$ and $k_2 > k_1$</p> <p>N.B.: It is impossible to have a sum of two growths or a growth–decay curve.</p>
B and C	$A(t) = A_A^0 \left(\frac{k_2 \epsilon_C - k_1 \epsilon_B}{\epsilon_A} \right) \left(\frac{1}{k_1 - k_2} \right) e^{-k_1 t}$ $+ A_A^0 \left(\frac{\epsilon_B - \epsilon_C}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right)$ $A(0) = 0$ $A(\infty) = A_A^0 \left(\frac{\epsilon_C}{\epsilon_A} \right)$	<p>Sum of two growths if $\epsilon_B > \epsilon_C$, $k_2 > k_1$, and $\frac{k_2}{k_1} > \frac{\epsilon_B}{\epsilon_C}$</p> <p>Growth–decay if $\epsilon_B > \epsilon_C$, $k_2 > k_1$, and $\frac{k_2}{k_1} < \frac{\epsilon_B}{\epsilon_C}$</p> <p>or if $\epsilon_B > \epsilon_C$, $k_1 > k_2$, and $\frac{k_2}{k_1} < \frac{\epsilon_B}{\epsilon_C}$</p> <p>Sigmoidal growth if $\epsilon_B < \epsilon_C$, $k_1 > k_2$, and $\frac{k_2}{k_1} > \frac{\epsilon_B}{\epsilon_C}$</p> <p>or if $\epsilon_B < \epsilon_C$, $k_1 < k_2$, and $\frac{k_2}{k_1} > \frac{\epsilon_B}{\epsilon_C}$</p> <p>N.B.: It is impossible to have a sum of two decays.</p>

steps in the reaction sequence for either of these growth–decay curves when these curves are treated separately by data fits. The assignments can be made only when the results of the two data fits are compared. This apparent ambiguity has been stated by others (6, 14) in terms of two solutions satisfying an equation of the form of eq. [2b] and interchanging the values of the rate constants in eq. [2b]. It should be made clear that the operation of transposing k_1 and k_2 in eq. [2b] is different from and not to be confused with the functional analysis given previously in which the ratio of k_2 to k_1 in eq. [2b] is varied. In chemical terms, a given growth–decay kinetic curve may correspond to either of two situations: (i) a fast formation of a strongly absorbing intermediate in the first step followed by its slow decomposition in the second step, or (ii) a slow formation of a weakly absorbing intermediate in the first step followed by its rapid decomposition in the second step.

It can be shown that the same ambiguity holds true for decay–growth curves, and hence we are led to the statement given in Theorem 4.

Theorem 4. For growth–decay and decay–growth functions of the forms of eqs. [8] and [11] it is impossible to assign unambiguously the rate constant parameters to the particular steps in the reaction sequence solely on the basis of the results of a single data fit according to these equations.

Determining the rate constant – reaction step correspondence

External chemical information

An obvious method to relieve the ambiguity stated in Theorem 4 is to obtain one of the rate constants in eq. [1], either k_1 or k_2 , from a separate experiment. The easier rate constant to obtain is k_1 , since this is immediately derived from the single exponential decay of species A. However, as already noted, the methodology of conducting the time-resolved experiment may preclude such a solution. If all species in a given chemical system have overlapping absorptions, then it would be difficult to obtain unique absorbance–time functions for species A and B. This is especially true if species A and B either have similar structures so that they are not distinguishable by different monitoring absorption bands or have weak chromophores altogether. Only a fortuitous situation in which species A and B have unique UV-vis absorption bands will permit this method to work. Detection by infrared absorption spectroscopy, on the other hand, is more powerful in this regard, as it is more likely to distinguish chemical species from unique IR absorption bands than it is from UV-vis ones, particularly in solution-phase experiments.

Yamasaki et al. (7, 8) recently proposed a new integrated profiles method to handle simultaneous analysis of the absorbance–time functions for species A and B given in eq. [1],

Table 4. Absorbance–time functions for observing three chemical species in eq. [1].

Case	Absorbance–time function	Function type	
A, B, and C	$A(t) = A_A^0 \left[1 + \left(\frac{k_2 \varepsilon_C - k_1 \varepsilon_B}{\varepsilon_A} \right) \left(\frac{1}{k_1 - k_2} \right) \right] e^{-k_1 t}$ $+ A_A^0 \left(\frac{\varepsilon_B - \varepsilon_C}{\varepsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0 \left(\frac{\varepsilon_C}{\varepsilon_A} \right)$ $A(0) = A_A^0$ $A(\infty) = A_A^0 \left(\frac{\varepsilon_C}{\varepsilon_A} \right)$	<p>Sum of two decays if</p> $\varepsilon_B < \varepsilon_C, k_1 < k_2, \frac{\varepsilon_A}{\varepsilon_C} > \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>Growth–decay if</p> $\varepsilon_B > \varepsilon_C, k_1 > k_2, \text{ and } \frac{\varepsilon_A}{\varepsilon_C} < \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>or if</p> $\varepsilon_B > \varepsilon_C, k_1 < k_2, \frac{\varepsilon_A}{\varepsilon_C} > \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>and $\frac{k_2}{k_1} > \frac{\varepsilon_B}{\varepsilon_C}$</p> <p>Sum of two growths if</p> $\varepsilon_B > \varepsilon_C, k_1 < k_2, \frac{\varepsilon_A}{\varepsilon_C} < \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>and $\frac{k_2}{k_1} > \frac{\varepsilon_B}{\varepsilon_C}$</p>	<p>Decay–growth if</p> $\varepsilon_B < \varepsilon_C, k_1 > k_2, \frac{\varepsilon_A}{\varepsilon_C} < \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>and $\frac{k_2}{k_1} < \frac{\varepsilon_B}{\varepsilon_C}$</p> <p>or if</p> $\varepsilon_B < \varepsilon_C, k_1 > k_2, \frac{\varepsilon_A}{\varepsilon_C} > \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>and $\frac{k_2}{k_1} < \frac{\varepsilon_B}{\varepsilon_C}$</p> <p>Sigmoidal growth if</p> $\varepsilon_B < \varepsilon_C, k_1 < k_2, \text{ and } \frac{\varepsilon_A}{\varepsilon_C} < \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$ <p>Sigmoidal decay if</p> $\varepsilon_B > \varepsilon_C, k_1 > k_2, \text{ and } \frac{\varepsilon_A}{\varepsilon_C} > \frac{\frac{k_2 - \varepsilon_B}{k_1} - \varepsilon_C}{\frac{k_2}{k_1} - 1}$

Table 5. Absorbance–time functions for observing three chemical species in eq. [1] at isobestic points.

Isobestic point	Absorbance–time function	Function type
$\epsilon_A = \epsilon_B$	$A(t) = A_A^0 \left[1 + \left(\frac{k_2 \epsilon_C - k_1 \epsilon_B}{\epsilon_B} \right) \left(\frac{1}{k_1 - k_2} \right) \right] e^{-k_1 t}$ $+ A_A^0 \left(\frac{\epsilon_B - \epsilon_C}{\epsilon_B} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0 \left(\frac{\epsilon_C}{\epsilon_B} \right)$	Sigmoidal decay if $\epsilon_B > \epsilon_C$ and $k_1 > k_2$ or if $\epsilon_B > \epsilon_C$ and $k_1 < k_2$ Sigmoidal growth if $\epsilon_B < \epsilon_C$ and $k_1 > k_2$ or if $\epsilon_B < \epsilon_C$ and $k_1 < k_2$
$\epsilon_A = \epsilon_C$	$A(t) = A_A^0 \left[1 + \left(\frac{k_2 \epsilon_C - k_1 \epsilon_B}{\epsilon_C} \right) \left(\frac{1}{k_1 - k_2} \right) \right] e^{-k_1 t}$ $+ A_A^0 \left(\frac{\epsilon_B - \epsilon_C}{\epsilon_C} \right) \left(\frac{k_1}{k_1 - k_2} \right) e^{-k_2 t} + A_A^0$	Growth–decay if $\epsilon_B > \epsilon_C$ and $k_1 > k_2$ or if $\epsilon_B > \epsilon_C$ and $k_1 < k_2$ Decay–growth if $\epsilon_B < \epsilon_C$ and $k_1 > k_2$ or if $\epsilon_B < \epsilon_C$ and $k_1 < k_2$
$\epsilon_B = \epsilon_C$	$A(t) = A_A^0 \left[1 - \frac{\epsilon_B}{\epsilon_A} \right] e^{-k_1 t} + A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right)$	Growth if $\epsilon_B > \epsilon_C$ Decay if $\epsilon_B < \epsilon_C$

provided that the condition of unique detection of the species is met. The strength of the method is that it is able to resolve nearly equally valued rate constants even when kinetic data may contain significant instrumentation noise.

Absorption coefficient method

A method based on the magnitude and variation of the absorption coefficient of intermediate B has been reported as a way of deciding which rate constant corresponds to which step in the reaction sequence (14). It is based on comparing the set of pre-exponential factors in an equation of the form of eq. [11] with that obtained when k_1 and k_2 are transposed. Taking the general case of observing all three species simultaneously, we have two forms of the absorbance–time equation given in Table 4. Equation [12] applies when k_1 is assigned to step 1 and k_2 is assigned to step 2.

$$[12] \quad A(t) = ae^{-k_1 t} + be^{-k_2 t} + c$$

where $a = A_A^0 [1 + (k_2 \epsilon_C - k_1 \epsilon_B) / \epsilon_A (k_1 - k_2)]$, $b = A_A^0 k_1 (\epsilon_B - \epsilon_C) / \epsilon_A (k_1 - k_2)$, and $c = A_A^0 (\epsilon_C / \epsilon_A)$. Equation [13] applies when k_1 is assigned to step 2 and k_2 is assigned to step 1.

$$[13] \quad A(t) = a' e^{-k_2 t} + b' e^{-k_1 t} + c'$$

where $a' = A_A^0 [1 + (k_1 \epsilon_C' - k_2 \epsilon_B') / \epsilon_A' (k_2 - k_1)]$, $b' = A_A^0 k_2 (\epsilon_B' - \epsilon_C') / \epsilon_A' (k_2 - k_1)$, and $c' = A_A^0 (\epsilon_C' / \epsilon_A')$. Since either of these expressions may describe a given experimental kinetic trace, the value of the absorbance, $A(t)$, is the same in each case. Hence, equating eqs. [12] and [13] results in

$$[14a] \quad a = b'$$

$$[14b] \quad b = a'$$

$$[14c] \quad c = c'$$

From eqs. [14a]–[14c] we have the following equalities relating absorption coefficients:

$$[15a] \quad \epsilon_A' = \epsilon_A$$

$$[15b] \quad \epsilon_B' = \epsilon_A + \frac{k_1}{k_2} (\epsilon_B - \epsilon_A)$$

$$[15c] \quad \epsilon_C' = \epsilon_C$$

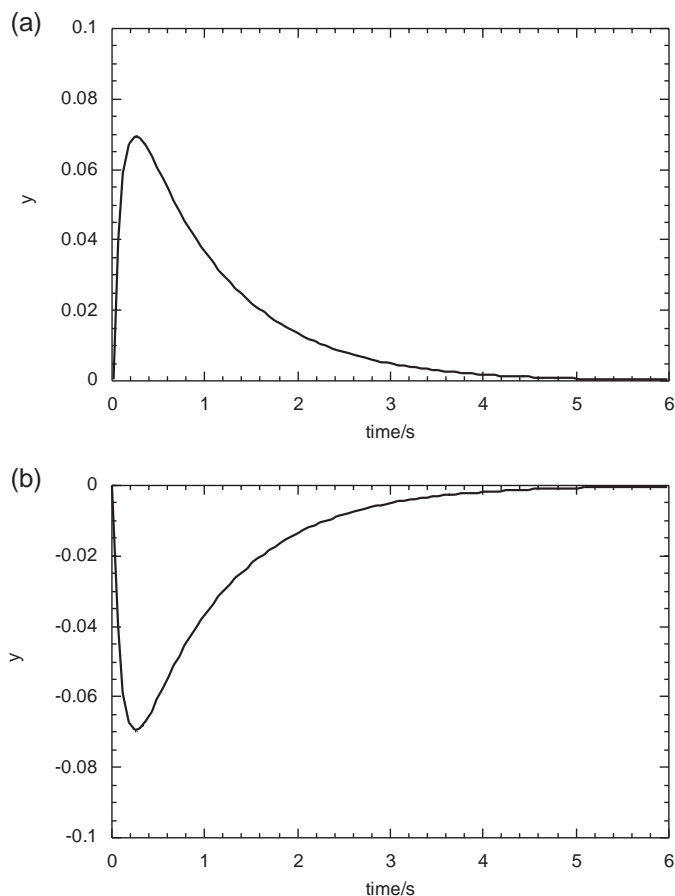
With prior knowledge of the values of the absorption coefficients of reactant A and product C, two possible values for the absorption coefficient of intermediate B, ϵ_B and ϵ_B' , may be obtained corresponding to the two sets of rate constant assignments. As noted previously (6, 14), the “correct” value of the absorption coefficient for B may be decided immediately on the basis of structural assignments if a stable analogue of B is known. Otherwise, the assignment will be based on whether or not the absorption coefficient varies with the ratio k_1/k_2 as given by eq. [15b]. This necessarily means examining other kinetic traces of the form of eq. [11] recorded at different values of k_1/k_2 . A correct value of the absorption coefficient is one that is independent of k_1/k_2 or k_2/k_1 . As written, eq. [15b] implies that ϵ_B is the correct value, since it is independent of k_1/k_2 ; whereas ϵ_B' is the incorrect value, since it is linearly dependent on this ratio. The reverse statement is true if eq. [15b] is solved in terms of ϵ_B as the dependent variable so that in this case ϵ_B is linearly

Table 6. Summary of functional analysis on model equation $y = a[\exp(-bt) - \exp(-ct)]$.

Pre-exponential factor	Relative rate constant magnitude	Function type	First part of curve		Second part of curve	
			Assigned rate constant	Function type	Assigned rate constant	Function type
$a > 0$	$c > b$	Growth-decay	c	Growth	b	Decay
$a < 0$	$b > c$	Growth-decay	b	Growth	c	Decay
$a > 0$	$b > c$	Decay-growth	b	Decay	c	Growth
$a < 0$	$c > b$	Decay-growth	c	Decay	b	Growth

Table 7. Summary of functional analysis on model equation $y = a \exp(-\alpha t) - b \exp(-\beta t) + c$.

Pre-exponential factor	Relative rate constant magnitude	Function type	First part of curve		Second part of curve	
			Assigned rate function	Function type	Assigned rate function	Function type
$a > 0$ and $b > 0$	$\alpha > \beta$	Decay-growth	α	Decay	β	Growth
$a < 0$ and $b < 0$	$\beta > \alpha$	Decay-growth	β	Decay	α	Growth
$a < 0$ and $b > 0$	$\alpha > \beta$	Sum of two growths	α	Growth	β	Growth
$a < 0$ and $b > 0$	$\beta > \alpha$	Sum of two growths	β	Growth	α	Growth
$a > 0$ and $b < 0$	$\alpha > \beta$	Sum of two decays	α	Decay	β	Decay
$a > 0$ and $b < 0$	$\beta > \alpha$	Sum of two decays	β	Decay	α	Decay
$a < 0$ and $b < 0$	$\alpha > \beta$	Growth-decay	α	Growth	β	Decay
$a > 0$ and $b > 0$	$\beta > \alpha$	Growth-decay	β	Growth	α	Decay

Fig. 2. Plot of functions (a) $y = 0.1[\exp(-t) - \exp(-10t)]$ and (b) $y = 0.1[\exp(-10t) - \exp(-t)]$.

dependent on k_2/k_1 and ϵ_B' is not. An alternative formalism of distinguishing ϵ_B and ϵ_B' , which is more practical, is to express each in terms of the pre-exponential coefficients a , b , and c that would be obtained from nonlinear least-squares analysis. The relationships are given by eqs. [16a] and [16b].

$$[16a] \quad \epsilon_B = \frac{\epsilon_A}{A_A^0} \left[b \left(1 - \frac{k_2}{k_1} \right) + c \right]$$

$$[16b] \quad \epsilon_B' = \frac{\epsilon_A}{A_A^0} \left[a \left(1 - \frac{k_1}{k_2} \right) + c \right]$$

It may be verified that eq. [16a] is invariant to the ratio of rate constants whereas eq. [16b] is not when ϵ_B is the correct absorption coefficient, and that the reverse is true when ϵ_B' is the correct absorption coefficient (see Appendix B).

Absorbance extremum method

If the transformations A to B and B to C in eq. [1] are catalyzed by some species Q such that the concentration of catalyst, [Q], is invariant with time during the course of the kinetic experiment, then the absorption-time functions for A, B, and C given by eqs. [4a]–[4c] still apply. Such an experimental constraint represents the well-documented pseudo-first-order condition. In general, the rate constants k_1 and k_2 will be some functions of the concentration of catalyst; for example, $k_1 = f([Q])$ and $k_2 = g([Q])$. The subscripts 1 and 2 refer to the first and second steps, respectively, so that the first step is associated with f and the second step is associated with g . Conversely, the functional assignments to the rate constants may be reversed so that $k_2 = f([Q])$ and $k_1 = g([Q])$. To resolve which assignment pertains to an experimental situation, the absorbance extremum is monitored as a function of catalyst concentration [Q]. The functional behaviour of the extremum with catalyst concentration will be

different for the two possible rate constant assignments. In the case of growth–decay curves the absorbance extremum is a maximum, and in the case of decay–growth curves it is a minimum. An important experimental criterion that must be met for this method to work is that the initial absorbance of species A must be kept constant in all experiments constituting the range of catalyst concentrations examined. This ensures that the correct values of the absorption maxima or minima are recorded from the collected growth–decay or decay–growth curves, respectively. Any artifacts or detractors from this criterion will result in spoiled data. Since growth–decay curves are more often encountered experimentally, we will now illustrate the method for these functions obtained under various experimental situations involving species B.

The case when B is observed uniquely

From eq. [6a], the absorption maximum of species B takes the form

$$[17] \quad A_B^{\max} = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{f}{g-f} \right) \left[\left(\frac{g}{f} \right)^{-f/(g-f)} - \left(\frac{g}{f} \right)^{-g/(g-f)} \right]$$

where A_B^{\max} is a function of [Q], when the assignments $k_1 = f([Q])$ and $k_2 = g([Q])$ are made. If the functional assignments of k_1 and k_2 are transposed so that $k_2 = f([Q])$ and $k_1 = g([Q])$, then the resultant analogous expression is given by

$$[18] \quad A_B^{\max} = A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{g}{f-g} \right) \left[\left(\frac{f}{g} \right)^{-g/(f-g)} - \left(\frac{f}{g} \right)^{-f/(f-g)} \right]$$

$$= A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{g}{g-f} \right) \left[\left(\frac{g}{f} \right)^{-f/(g-f)} - \left(\frac{g}{f} \right)^{-g/(g-f)} \right]$$

where the second step is associated with f and the first step is associated with g . A closer examination of eqs. [17] and [18] indicates that the expressions are distinguishable by the factors $f/(g-f)$ and $g/(g-f)$. Hence, it is possible to assign a rate constant to a particular step in the sequence on the basis of its functional dependence on catalyst concentration. This does *not* imply that the calculated rate constant parameters obtained from a *single* growth–decay curve may be assigned to a specific reaction step (see Theorem 4). To distinguish which rate constant is associated with which step in the reaction sequence, a *collective* analysis must be performed on all growth–decay curves obtained over the catalyst concentration range examined. The methodology is as follows. The $(t_i, A_{B,i})$ data points from a kinetic trace obtained at a given catalyst concentration are first fitted to the model eq. [8] using a nonlinear least-squares algorithm. Then, the absorption maximum of this trace is determined from the calculated parameters at $t = (1/(c-b)) \ln(c/b)$ according to $y_m = a[(c/b)^{-b/(c-b)} - (c/b)^{-c/(c-b)}]$. Note that $y_m > 0$ when $a > 0$ and $c > b$, or when $a < 0$ and $b > c$. The process is repeated for other kinetic traces recorded at several catalyst concentrations until a set of data points $([Q]_i, y_{m,i})$ is obtained. The functions given by eqs. [17] and [18] can be constructed once the functional forms for f and g are known. The data points $([Q]_i, y_{m,i})$ are then plotted and matched with the appropriate curve. A match with eq. [17], for example, indi-

cates that the first step in the reaction is associated with the rate constant having a catalyst dependence given by $f([Q])$, and the second step is associated with the rate constant having a catalyst dependence given by $g([Q])$.

We illustrate the method with two numerical examples. Suppose we have a chemical system described by eq. [1] such that the first step is invariant to catalyst concentration while the second step obeys a linear relationship with catalyst concentration. From our growth–decay curves for species B we obtain parameters b and c but cannot assign these to the particular steps in the reaction scheme on the basis of any one curve. Hence, we have either case (i) $b = f([Q]) = D_1$ and $c = g([Q]) = D_2 + D_3[Q]$ or case (ii) $c = f([Q]) = D_1$ and $b = g([Q]) = D_2 + D_3[Q]$, where the constants D_1 , D_2 , and D_3 would be known from separate experiments. To overcome the ambiguity, plots according to the forms of eqs. [17] and [18] are made and compared with the actual $y_{m,i}$ versus $[Q]_i$ plot to determine which of the above cases is applicable. Figures 3a and 3b illustrate the functions given by eqs. [17] and [18] using the definitions of f and g given in cases (i) and (ii). The method also works when the rate constants b and c are independent of catalyst concentration; i.e., we have either case (iii) $b = f([Q]) = D_1$ and $c = g([Q]) = D_2$ or case (iv) $c = f([Q]) = D_1$ and $b = g([Q]) = D_2$. Figures 4a and 4b illustrate the functions given by eqs. [17] and [18] using the definitions of f and g given in cases (iii) and (iv).

Cases when B and A, B, and C, and B, C, and A are observed together

From Tables 3 and 4 the functions describing growth–decay curves for these three cases may be conveniently described by the relation

$$[19] \quad y = a \exp(-k_1 t) - b \exp(-k_2 t) + c$$

where

$$a = A_A^0 \left[1 - \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right) \right]$$

$$b = -A_A^0 \left(\frac{\epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right)$$

and $c = 0$ apply to observing B and A together;

$$a = A_A^0 \left[\left(\frac{k_2 \epsilon_C - k_1 \epsilon_B}{\epsilon_A} \right) \left(\frac{1}{k_1 - k_2} \right) \right]$$

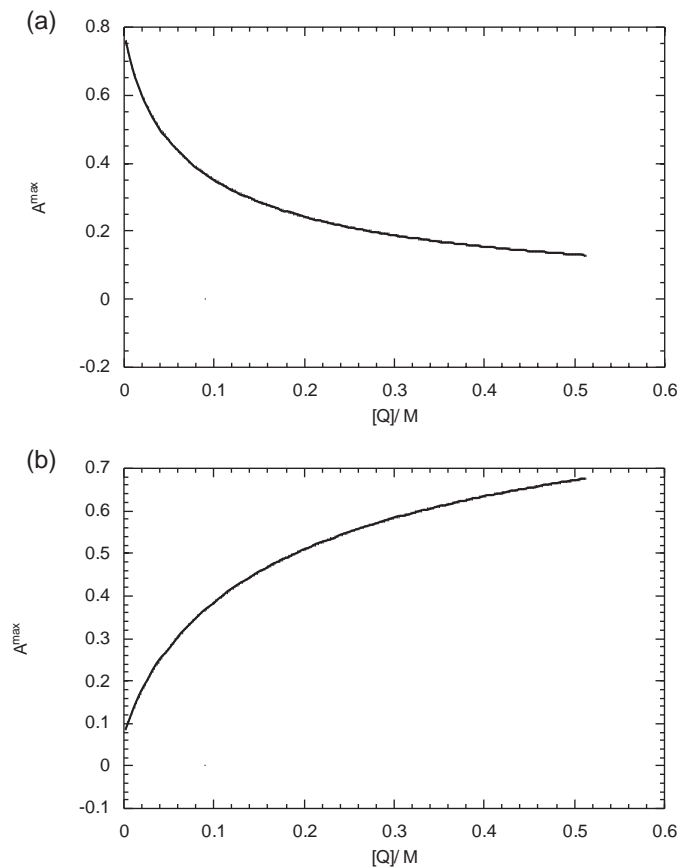
$$b = A_A^0 \left(\frac{\epsilon_C - \epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right)$$

and $c = A_A^0 (\epsilon_C/\epsilon_A)$ apply to observing B and C together; and

$$a = A_A^0 \left[1 + \left(\frac{k_2 \epsilon_C - k_1 \epsilon_B}{\epsilon_A} \right) \left(\frac{1}{k_1 - k_2} \right) \right]$$

$$b = A_A^0 \left(\frac{\epsilon_C - \epsilon_B}{\epsilon_A} \right) \left(\frac{k_1}{k_1 - k_2} \right)$$

Fig. 3. Plot of (a) eq. [17] and (b) eq. [18] with $f([Q]) = D_1 = 10$, $g([Q]) = D_2 + D_3[Q] = 1 + 100[Q]$, and $A_A^0(\epsilon_B/\epsilon_A) = 1$. If the data fit this equation then (a) the function f corresponds to the first step, and the function g corresponds to the second step; (b) the function g corresponds to the first step, and the function f corresponds to the second step.



and $c = A_A^0(\epsilon_C/\epsilon_A)$ apply to observing B, C, and A together. The absorbance maximum occurs at

$$[20] \quad t = \frac{1}{k_2 - k_1} \ln \left(\frac{bk_2}{ak_1} \right)$$

and has a value given by

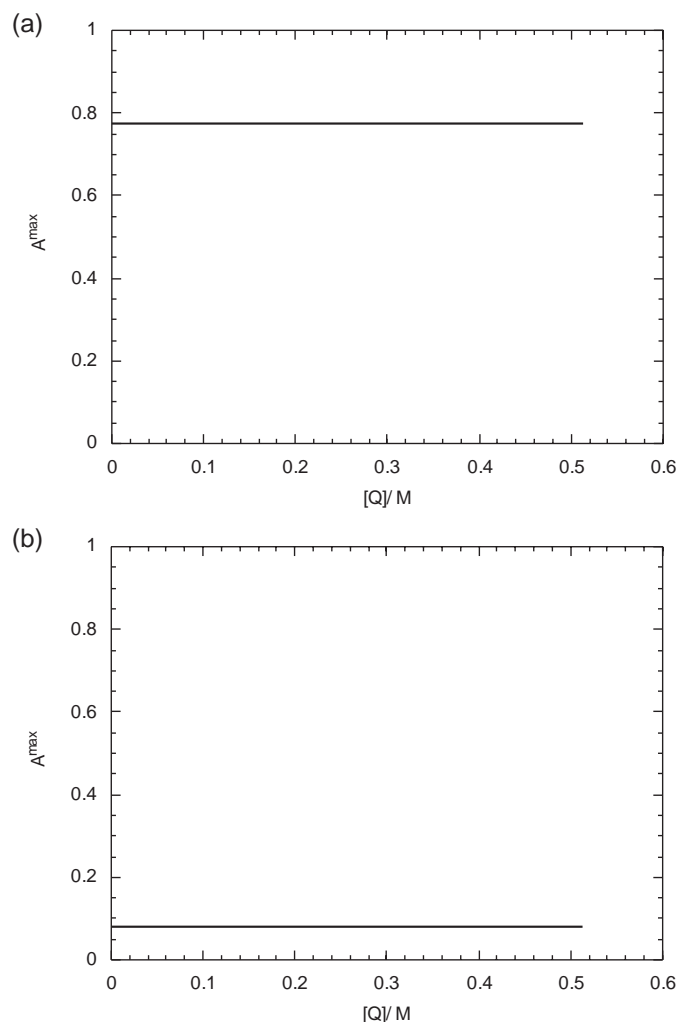
$$[21] \quad y_m = a \left(\frac{bk_2}{ak_1} \right)^{-k_1/(k_2 - k_1)} - b \left(\frac{bk_2}{ak_1} \right)^{-k_2/(k_2 - k_1)} + c$$

where the coefficients a and b are also functions of k_1 and k_2 , as given by the relationships defined above depending on what chemical species are observed. The functional form of the absorbance maximum with catalyst concentration is given by

$$[22] \quad A^{\max} = a \left(\frac{bg}{af} \right)^{-f/(g-f)} - b \left(\frac{bg}{af} \right)^{-g/(g-f)} + c$$

with the assignment $k_1 = f([Q])$ and $k_2 = g([Q])$ made in the appropriate expressions for the pre-exponential parameters; and by

Fig. 4. Plot of (a) eq. [17] and (b) eq. [18] with $f([Q]) = D_1 = 10$, $g([Q]) = D_2 = 1$, and $A_A^0(\epsilon_B/\epsilon_A) = 1$. If the data fit this equation then (a) the function f corresponds to the first step, and the function g corresponds to the second step; (b) the function g corresponds to the first step, and the function f corresponds to the second step.



$$[23] \quad A^{\max} = a \left(\frac{bf}{ag} \right)^{-g/(f-g)} - b \left(\frac{bf}{ag} \right)^{-f/(f-g)} + c$$

with the assignment $k_1 = g([Q])$ and $k_2 = f([Q])$.

Summary

A detailed account of the forms of the absorbance–time functions of the unimolecular consecutive two-step reaction sequence is given. The problem of distinguishability of rate constants is discussed with particular emphasis on the rate constant – reaction step ambiguity. A general method based on the functional form of the absorption extremum with catalyst concentration is proposed as a means to overcome this ambiguity in rate constant assignments. Of particular interest to biochemists, enzymologists, and medicinal chemists is the

application of this new method to the resolution of consecutive pseudo-first-order rate constants which are functions of pH. Chemical reactions obeying mechanisms of this type will then be described by simultaneous pH-rate profiles. Work is currently in progress on the theoretical and experimental verification of this new method for such chemical systems.

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Appendix A

In this appendix a proof is given for the relationship

$$[A1] \quad \lim_{n \rightarrow 1} \left\{ \frac{1}{n-1} [n^{-1/(n-1)} - n^{-n/(n-1)}] \right\} = e^{-1}$$

Proof: Since substitution of $n = 1$ leads to an indeterminate form, the expression is rewritten as a rational function and l'Hôpital's rule (16) is applied as shown by the sequence of steps below.

$$\begin{aligned}
 [A2] \quad & \lim_{n \rightarrow 1} \left\{ \frac{1}{n-1} [n^{-1/(n-1)} - n^{-n/(n-1)}] \right\} \\
 &= \lim_{n \rightarrow 1} \left\{ \frac{\left(\frac{1}{n}\right)^{-1/(n-1)} - \left(\frac{1}{n}\right)^{-n/(n-1)}}{n-1} \right\} \\
 &= \lim_{n \rightarrow 1} \left\{ \frac{\left(\frac{1}{n}\right)^{-1/(n-1)} \left[\frac{\ln n + \frac{1}{n} - 1}{(n-1)^2} \right] - \left(\frac{1}{n}\right)^{-n/(n-1)} \left[\frac{\ln n + 1 - n}{(n-1)^2} \right]}{1} \right\} \\
 &= \lim_{n \rightarrow 1} \left\{ \left(\frac{1}{n}\right)^{1/(n-1)} \left[\frac{\ln n}{n(n-1)} \right] \right\} \\
 &= \lim_{n \rightarrow 1} \left\{ \left(\frac{1}{n}\right)^{1/(n-1)} \right\} \lim_{n \rightarrow 1} \left\{ \frac{\ln n}{n(n-1)} \right\}
 \end{aligned}$$

Each of the limits above is then evaluated separately. The left-hand limit is evaluated as follows. Letting $z = n^{-1/(n-1)}$ and taking natural logarithms of both sides leads to

$$\ln z = -\frac{\ln n}{n-1}$$

Then,

$$[A3] \quad \lim_{n \rightarrow 1} \ln z = -\lim_{n \rightarrow 1} \left\{ \frac{\ln n}{n-1} \right\} = -\lim_{n \rightarrow 1} \left\{ \frac{\frac{1}{n}}{1} \right\} = -1$$

after again applying l'Hôpital's rule, since substitution of $n = 1$ leads to an indeterminate form. Hence,

$$[A4] \quad \lim_{n \rightarrow 1} z = \lim_{n \rightarrow 1} \{n^{-1/(n-1)}\} = e^{-1}$$

The right-hand limit of eq. [A2] is evaluated with a final application of l'Hôpital's rule to yield

$$[A5] \quad \lim_{n \rightarrow 1} \left\{ \frac{\ln n}{n(n-1)} \right\} = \lim_{n \rightarrow 1} \left\{ \frac{\frac{1}{n}}{2n-1} \right\} = 1$$

Substitution of these results into eq. [A2] completes the proof.²

²A referee has suggested an alternative solution which involves substituting $n = 1 + \epsilon$ in eq. [A1] and evaluating the limit as $\epsilon \rightarrow 0$. The resultant limit is $\lim_{\epsilon \rightarrow 0} (1 + \epsilon)^{-(1+\epsilon)/\epsilon}$ which can be evaluated to give e^{-1} using the same sequence of steps in arriving at eq. [A4] from eq. [A3].

Appendix B

Equations [16a]–[16b] are obtained by first rearranging the expressions for the pre-exponential factors b and b' , respectively, and then substituting a for b' in the case of eq. [16b]. If ϵ_B is the correct absorption coefficient for the intermediate B then eq. [16a] becomes an identity upon substitution of the expression for b , thus making ϵ_B independent of k_1/k_2 . On the other hand, eq. [16b] reduces to

$$[\text{B1}] \quad \epsilon_B' = \epsilon_A + (\epsilon_B - \epsilon_A) \frac{k_1}{k_2}$$

upon substitution of the expression for a , thus making ϵ_B' linearly dependent on k_1/k_2 . Note that eq. [B1] is identical to eq. [15b]. An assignment of the absorption coefficient of B to ϵ_B implies that the first step in the sequential mechanism corresponds to rate constant k_1 and that the second step corresponds to rate constant k_2 . If ϵ_B' is the correct absorption coefficient for the intermediate B, then eq. [16a] reduces to

$$[\text{B2}] \quad \epsilon_B = \epsilon_A + (\epsilon_B' - \epsilon_A) \frac{k_2}{k_1}$$

upon substitution of b with the expression for a' , thus making ϵ_B linearly dependent on k_2/k_1 . On the other hand, eq. [16b] becomes an identity upon substitution of a with the expression for b' , thus making ϵ_B' independent of k_2/k_1 . An assignment of the absorption coefficient of B to ϵ_B' implies that the first step in the sequential mechanism corresponds to rate constant k_2 and that the second step corresponds to rate constant k_1 . It should be noted that eqs. [B1] and [B2] are identical upon rearrangement. Equation [B1] is written with ϵ_B' as the dependent variable while eq. [B2] is written with ϵ_B as the dependent variable.

In practice kinetic traces recorded at various k_1/k_2 ratios are fit to an expression of the form of eq. [11]. At this stage the rate constant subscript labels 1 and 2 are arbitrary. Having obtained the five parameters from nonlinear least-squares analysis, plots of $b(1 - k_2/k_1) + c$ versus k_1/k_2 and $a(1 - k_1/k_2) + c$ versus k_1/k_2 are then made. If the former plot shows no dependence on k_1/k_2 and the latter plot shows a linear dependence on k_1/k_2 , then the absorption coefficient of B is ϵ_B and k_1 and k_2 are assigned to the first and second steps, respectively. If, on the other hand, the former plot shows a hyperbolic dependence on k_1/k_2 (linear dependence on k_2/k_1) and the latter plot shows no dependence on k_1/k_2 , then the absorption coefficient of B is ϵ_B' , and k_1 and k_2 are assigned to the second and first steps, respectively.