Three Theorems for the Spectroscopic-Kinetic Analysis of Chemical Reactions

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Dedicated to Prof. Dr. H.-L. Schmidt on the occasion of his 65th birthday

The results obtained in the last 25–30 years for the spectroscopic-kinetic analysis of reaction systems using absorbance (A) diagrams and the method of formal integration are summarised in three theorems.

Theorem 1: A reaction mechanism can be experimentally disproved but not proved.

Theorem 2: Two strictly linear reaction systems having the same number of linearly independent reaction steps can not be distinguished from each other by purely spectroscopic means.

Theorem 3: Thermally controlled reaction systems which consist of two linearly independent reaction steps – one step of which is at least a reaction of second order – can not be distinguished from each other by purely spectroscopic means if their eigenvalues have the same functional dependence on the initial concentrations.

The advantages of the formalism presented are demonstrated using the photoreduction of anthraquinone-2-carbonic acid in alkaline methanolic solution.

Introduction

The main task of kinetic analysis is the clearing up of the mechanisms of reactions. In principle it is always possible to compute numerically the course of a reaction provided there is a known mechanism using estimated parameters. However, there is no direct way to determine a mechanism from experimental data. In practice, a mechanism is first set up intuitively, and then the functions belonging to this mechanism are developed. Finally, a check is carried out if these functions are in agreement with the measured data. If the computed and measured data disagree, the hypothetical mechanism has to be discarded and a new one is examined. If they correspond, the mechanism is still far from being proved since there are many mechanisms which can show the same functional development in the experimental data. In addition, the accuracy of the measurements is limited. These facts lead directly to the first theorem. Both other theorems refer to reaction systems studied UV-VIS spectroscopically.

The UV-VIS spectroscopy is the preferred method for kinetic analysis of reaction systems since UV-VIS measurements

- are easy to perform (a large number of suitable devices are on the market)
- the measurement accuracy is high and
- many absorbances can be measured as a function of time at several wavelengths (or spectra can even be recorded in terms of time dependence).

The basic equation for the evaluation of UV-VIS data is the generalized Lambert-Beer-Bouguer law [1, 2]. The methods presented in the following can always be used if the applied spectroscopic measurement obeys a low being analogous to the Lambert-Beer-Bouguer equation. These correlations may be found in fluorescence, IR, ORD, CD and NMR spectroscopy [2].

Theory and Results

1. Theorem 1 on Arbitrary Reaction Systems

The well known hypothesis of Bodenstein [1–3] can be applied to reaction systems in which intermediates are involved in very low concentrations. Here, mostly intermediates M_i are concerned whose analysis is difficult. The hypothesis of Bodenstein predicts that

i) the concentration m_i of M_i is negligible small $(m_i \approx 0)$,

ii) the change of m_i in time is negligible $(\dot{m}_i \approx 0)$.

Registered concentration-time curves generally supply more information about reaction mechanisms than absorbance-time curves obtained by the UV-VIS spectroscopy. Nevertheless, the proof of a reaction mechanism is not possible in both cases. For example, if the reaction $A \xrightarrow{k_1} C$ is considered, it can not be excluded that the reaction is running according to the mechanism $A \xrightarrow{k_1} B \xrightarrow{k_2} C$ provided that $k_2 \gg k_1$. Thus, intermediates can be involved in very low concentrations whose determination is difficult. Although there are more and more sophisticated methods for the identification of chemical compounds and for the registration of concentration-time data, it can only be demonstrated that an experiment does not conflict with a hypothetical model because of

Theorem 1:

A reaction mechanism can be experimentally disproved but not proved.

This theorem is applicable to chemical, biochemical and photochemical reactions.

2. Theorem 2 on Linear Reaction Systems

Chemical and photochemical reaction systems in solution are preferentially investigated by UV-VIS spectroscopic techniques. The collected data can be evaluated by using the "multiwavelength analysis method". Here, absorbance (A) diagrams and formal integration can be brought in [1, 2].

For the following, the terms "linar reaction systems" and "linearly independent reactions" are important. Linear reaction systems consist by definition of first order reaction steps. The thermally controlled reaction systems $A \rightarrow B$, $A \rightarrow B \rightarrow C$ or $A \rightarrow B \rightleftarrows C$ are examples of this. Linearly independent reactions are independent of reaction order. Each reaction mechanism consists of a distinct number (s) of linearly independent reaction steps. For example, the system $A \rightleftarrows B + C$ consists of only one linearly independent reaction $(s = 1)$. This reaction can be described by a single independent degree of advancement symbolized generally by a capital letter X_i $(i = 1 \ldots s)$. Although the reaction system $A \rightleftarrows B + C$ consists of two reaction steps being of first and second order, respectively, the system can be described by X_1 alone [1, 2].

Analogously, the system $A \rightleftarrows B \rightleftarrows C + D$ can be characterizied by X_1 and X_2 $(s = 2)$.

For the most part, the elementary chemical reaction steps of which the mechanism consists are of first or second order. The following theorem 2 refers to spectroscopically studied systems consisting only of reactions of the first order, and theorem 3 refers to systems in which reactions of second order are involved. For the comprehension of theorems 2 and 3 the basic equation for the relationship between absorbance (A) and degree of advancement (X) is required [2, 4]:

$$A = A_0 + \mathbf{Q} X \tag{1}$$

with

$$A = \begin{pmatrix} A_1 \\ \vdots \\ A_s \end{pmatrix}, \quad A_0 = \begin{pmatrix} A_{10} \\ \vdots \\ A_{s0} \end{pmatrix},$$

$$\mathbf{Q} = \begin{pmatrix} Q_{11} & \cdots & Q_{1s} \\ \vdots & & \vdots \\ Q_{s1} & \cdots & Q_{ss} \end{pmatrix}, \quad X = \begin{pmatrix} X_1 \\ \vdots \\ X_s \end{pmatrix}.$$

Here, the vector A contains the absorbances of s (appropriate) wavelengths $(\lambda = 1, \ldots, s)$ at time t. A_0 contains the absorbances refering to time $t = 0$. X is composed of s linearly independent degrees of advancement describing the reaction mechanism. The matrix $\mathbf{Q}$ contains only constants. In general, the coefficients Q_{ij} consist of the product of the cell pathlength and the stoichiometric sum of the molar absorption coefficients according to the (i) individual components of the j-th reaction step. For the most part, it is possible to find appropriate wavelengths so that $\mathbf{Q}$ becomes a regular matrix [1, 2]. Equation (1) is generally true and is independent of reaction order (the individual reactions can be of first or second order, for example).

A basic equation for linear reaction systems holds [5]:

$$\dot{X} = \dot{X}_0 + \mathbf{K} X. \tag{2}$$

$\mathbf{K}$ is a regular $s \cdot s$ matrix named Jacobian matrix:

$$\mathbf{K} = \begin{pmatrix} \dfrac{\partial \dot{X}_1}{\partial X_1} & \cdots & \dfrac{\partial \dot{X}_1}{\partial X_1} \\ \vdots & & \vdots \\ \dfrac{\partial \dot{X}_s}{\partial X_1} & \cdots & \dfrac{\partial \dot{X}_s}{\partial X_1} \end{pmatrix}. \tag{3}$$

Equation (2) is a Taylor expansion for $\dot{X}$ (starting from $t = 0$) being terminated with the first term. $\dot{X}_0$ is the

differential vector $\dot{X}$ for $t = 0$:

$$\dot{X} = \begin{pmatrix} \dot{X}_1 \\ \vdots \\ \dot{X}_s \end{pmatrix} \quad \text{and} \quad \dot{X}_0 = \begin{pmatrix} \dot{X}_1 \\ \vdots \\ \dot{X}_s \end{pmatrix}. \tag{4}$$

Equation (2) can be transformed into

$$\dot{X} = K(X - X_\infty) \tag{5}$$

with

$$X_\infty = -K^{-1} \dot{X}_0, \tag{6}$$

as shown in [5]. X_∞ is the vector X for $t \rightarrow \infty$. K^{-1} is the inverse matrix of K. Equations (5) and (2) are the main equations for calculating linear reaction systems.

The following equation can be derived by differentiating (1) with respect to time and using (5) [2, 4]:

$$\dot{A} = Z(A - A_\infty) \tag{7}$$

with

$$\dot{A} = \begin{pmatrix} \dfrac{dA_1}{dt} \\ \vdots \\ \dfrac{dA_s}{dt} \end{pmatrix} \quad \text{and} \quad A_\infty = \begin{pmatrix} A_{1\infty} \\ \vdots \\ A_{s\infty} \end{pmatrix},$$

where A_∞ contains the absorbance for $t \rightarrow \infty$. Z is a matrix which is related to the $(s \cdot s)$ Jacobian matrix K of the reaction system [4]:

$$Z = Q K Q^{-1} \tag{8}$$

(where Q^{-1} is the inverse matrix of Q).

Equation (7) is the basic differential equation for the evaluation of linear reaction systems using spectroscopic data.

From (8) it follows that Z and K are similar matrices [6]. Similar matrices have

- the same characteristic polynom
- the same eigenvalues
- the same sums of principal minors (for $s = 2$ the determinant and trace of Z are identical to those of K).

The solution of (7) contains s exponential terms, the coefficients of which can be determined by non-linear regression analysis [7–9]. However, a system of difference equations of first order or difference equations of higher order as well as recursion equations can also be used, as shown in [2, 4]. For $s = 1$ the methods according to Guggenheim [10], Kézdy et al. [11] and Swin-

bourne [12] are well known. The determination of the coefficients z_{ij} (of (7)), using the methods of formal integration, is here recommended [1, 2]. The eigenvalues of the reaction system can be obtained from z_{ij}. Thus, spectroscopic-kinetic analysis can be performed by combining (synchronized) absorbance-time values at s wavelengths. Practical examples for the application of this procedure are given in [1, 2, 13, 14].

Equation (7) is also useful for simulating absorbance-time curves. Applying the methods of Runge-Kutta-Merson [15] as well as Gear [16], it is possible to simulate the experimental curves on the basis of the determined values of z_{ij}. When the simulation is not in accordance with the experimental curves, the mechanism must be discarded. In the opposite case, the hypothetical mechanism is thus also confirmed.

In general, only the eigenvalues of the studied reaction system are obtained from the absorbance-time values. For example, considering the two reaction systems

$$A \rightarrow B \rightarrow C \quad \text{and} \quad A \rightarrow B, C \rightarrow D,$$
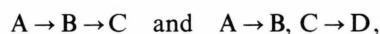
it is only possible to determine the two eigenvalues existing here for each mechanism. Moreover, both reaction systems can not at all be distinguished solely by spectroscopic-kinetic analysis. For this, additional information is always necessary. This information can be derived, for example, from chromatographic measurements. In order to generalise this situation two different linear reaction systems are considered. These systems may be described by the regular Jacobian matrices K and K'. Both matrices must have the same rank s, and all eigenvalues of K and K', respectively, must be different. With these prerequisites a transformation matrix T exists which converts K' to K as shown in [5]:

$$K = T K' T^{-1}, \tag{9}$$

where T^{-1} is the inverse matrix of T.

It follows from this equation that each linear reaction system (index ') can be transformed into another linear system of the same rank. This also means that the system ' can be transformed into another system by an affine transformation. According to [5] one has

$$X = T X'. \tag{10}$$

The Jacobian matrices of the two systems are similar according to (9).

The transformation of the system containing the degrees of advancement (X) into the system of ab-

sorbances (A) is also an affine transformation according to (1). Therefore, without any additional information, it can not be recognized at a later date which reaction system originally existed. This correlation leads to (compare [5, 4])

Theorem 2:
Two strictly linear reaction systems having the same number of linearly independent reaction steps can not be distinguished from each other by purely spectroscopic means.

Or in another version:

Two strictly linear reaction systems whose Jacobian matrices have the same rank can not be distinghuised from each other by purely spectroscopic means.

"Strictly linear" means that all reaction steps are running monomolecularly. "Purely spectroscopic" means that the molar absorptivities are unknown and that additional information is missing (i.e. only absorbance-time curves are known).

Theorem 2 is also valid for "quasilinear photoreactions". The typical example in [17] demonstrates how quasilinear photoreactions can be evaluated as thermally controlled reactions of the first order if the reaction time is replaced by the time coordinate θ, defined by

$$d\theta = 1000 \frac{1 - 10^{-A'}}{A'} \cdot dt. \tag{11}$$

A' is the absorbance at the irradiation wavelength λ'. With (11) the absorbance differential equations of quasilinear photoreactions can be formally reduced to (7) if the time t is replaced by θ (it is essential for quasilinear photoreactions that the partial quantum yields are independent of concentrations; see als Section 2.1).

The problem of transforming linear reaction systems into each other was also treated in [18]. However, another mathematical algorithm was used there.

The advantages of the mathematical procedure applied in this paper are connected with more complex kinetic problems treated in 3.1. Two basic questions in
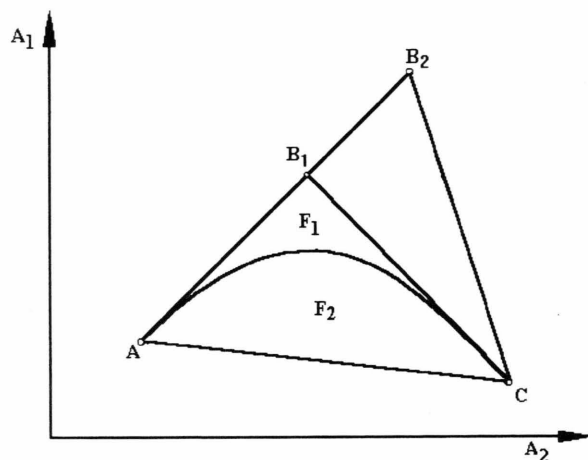

Fig. 1. Schematic absorbance (A) diagram A_1 vs. A_2 for $A \rightarrow B \rightarrow C$. The points A and C indicate the absorbance being obtained when the individual components are separate in solution ($A_{\lambda,i} = \ell \, \varepsilon_{\lambda,i} \, c_i$). The corresponding point for intermediate B can be point B_1 or B_2 (see text).

the past had led us to the algorithms presented here:

i) which information can be obtained solely by spectroscopic data (i.e. absorbance data),
ii) can kinetic measurement data be generally evaluated by linear regression methods?

The first question is closely related to the "absorbance (A)" and "absorbance difference quotient (ADQ)" diagrams [1, 2], and the second question to the "method of formal integration" [1, 2].

2.1. Measuring Example

The advantages of applying A diagrams and linear regression methods in kinetic analysis are subsequently demonstrated in the case of a photochemical reaction system.

Anthraquinones, such as anthraquinone-2-carbonic acid, can be photochemically reduced at 313 nm (irradiation wavelength λ') in oxygen-free alkaline methanolic solution according to the over-all mechanism $A \xrightarrow{hv} B \xrightarrow{hv} C$ ([1, 2, 19]):

anthraquinone-2-
carbonic acid (A)   radical anion (B)   dianion (C)
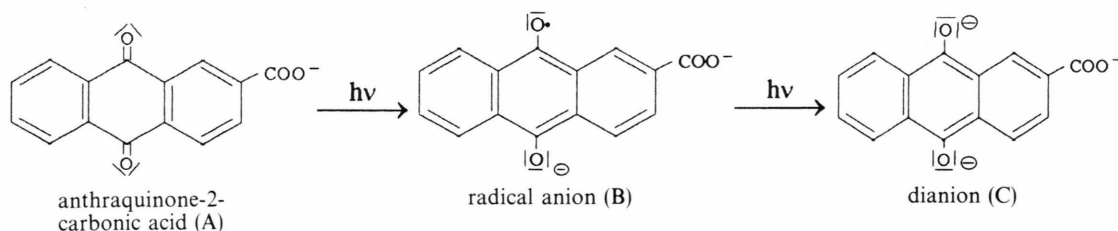
The existence of the radical anion B was confirmed by ESR measurements [19]. The measured absorbance-time values can be transformed into $A_\lambda - \theta$ data using (11) [2, 17].

In principle, the photoreduction can also run according to $A \rightleftarrows B \rightarrow C$, $A \rightarrow B \rightleftarrows C$ or $A \rightleftarrows B \rightleftarrows C$. However, in [19] it was shown with sufficient certainty that there is only one final product. This means that the mechanisms $A \rightarrow B \rightleftarrows C$ and $A \rightleftarrows B \rightleftarrows C$ can be excluded. Although the mechanism $A \rightleftarrows B \rightarrow C$ can not safely be discarded, the system $A \rightarrow B \rightarrow C$ is favoured here [19, 20]. Thus, the rate constants (k_i) can be used here instead of the eigenvalues (r_i) which are necessary for the general description of reaction systems (compare [1, 2, 4, 5]).

2.1.1 The Absorbance Diagram

In order to determine the information which can be obtained solely by absorbance data, the A diagram (A_1 vs. A_2) for the case $A \rightarrow B \rightarrow C$ is generally considered [2, 4, 5]. A typical curve for this system is shown in Figure 1. From this curve the ratio of k_i belonging to the mechanism

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$
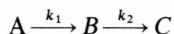
can be determined. Defining the ratio of k_i by κ one obtains for the photoreactions $A \xrightarrow{h\nu} B \xrightarrow{h\nu} C$ [1, 2]

$$\kappa = \frac{k_2}{k_1} = \frac{I_0 \, \varepsilon'_B \, \varphi_2^B}{I_0 \, \varepsilon'_A \, \varphi_1^A} = \frac{\varepsilon'_B \, \varphi_2^B}{\varepsilon'_A \, \varphi_1^A}$$

(ε'_A and ε'_B are the molar absorption coefficients of A and B at λ', φ_1^A and φ_2^B are the partial quantums yields of A and B; I_0 is the intensity of photolight at λ').

The points A and C in Fig. 1 correspond to the absorbances measured when only the components A or C are separate in solution ($A_{\lambda i} = \ell \, \varepsilon_{\lambda i} \, a_0$, ℓ = pathlength in cm, $\varepsilon_{\lambda i}$ = molar absorption coefficient of A or C, a_0 = initial concentration of A). The tangents to the points A and C (A represents the initial and C the end point of the reaction system) intersect in point B_1.

In the case $\kappa < 1$, the first reaction $A \xrightarrow{k_1} B$ is faster than the second one $B \xrightarrow{k_2} C$ ($k_1 > k_2$). If $\kappa < 1$, the point B_1 in Fig. 1 falls together with that point (B) which would be reached when the reaction $B \rightarrow C$ would not take place and the reaction $A \rightarrow B$ has finished. Then, the triangle AB_1C corresponds to the triangle ABC, which is an affinely distorted Gibbs

phase triangle of a system which consists of the components A, B, and C being combined by the stoichiometric equation

$$a + b + c = a_0.$$

In Fig. 1 F_1 indicates the area above the absorbance curve and F_2 that under he curve. If $\kappa < 1$ one has [5, 2]

$$\kappa = \frac{k_2}{k_1} = \frac{F_1}{F_2} < 1 \tag{a}$$

and for $A_\lambda(B)$ (with $\lambda = 1, 2$)

$$A_\lambda(B) = A_\lambda(B_1) = \ell \, \varepsilon_{\lambda B} \, a_0 \tag{b}$$

($\varepsilon_{\lambda B}$ = molar absorption coefficient of B).

However, if $\kappa > 1$ the point B falls together with the point B_2 (see Figure 1). B_2 can be constructed using the equation

$$\overline{AB_2} = \varrho \cdot \overline{AB_1}, \tag{c}$$

where ϱ is defined by the ratio

$$\varrho = \frac{\text{larger eigenvalue}}{\text{smaller eigenvalue}} > 1.$$

The following equation holds then true for κ:

$$\kappa = \frac{k_2}{k_1} = \frac{F_1}{F_2} > 1. \tag{d}$$

Here F_1 indicates the entire area above the curve AC in the triangle AB_2C (see Figure 1). Since $B = B_2$, one obtaines here for $A_\lambda(B)$

$$A_\lambda(B) = A_\lambda(B_2) = \ell \, \varepsilon_{\lambda B} \, a_0. \tag{e}$$

In general, it is not possible to decide by spectroscopic-kinetic analysis whether $\kappa > 1$ or $\kappa < 1$. Thus, when the tangents are constructed to the points A and C in the A diagram, and the ratio of the corresponding areas are determined as described above, either the value κ or its reciprocal value $1/\kappa$ is obtained.

The areas in the A diagram of antraquinone-2-carbonic acid lead to the following value for κ (compare Fig. 2)

$$\kappa = 6.0_7 \quad \text{or} \quad 1/\kappa = 6.0_7.$$

In some favourable cases it can be ascertained by means of A diagrams whether the first or second step is faster. In the case of the photoreduction the tangents to the points A and C lead to the point B ($B = B_1$, see Figure 2). Assuming $\kappa > 1$, the point B_2 can be calculated by (c) using $\varrho = 6.0_7$. Constructing B_2 in this way
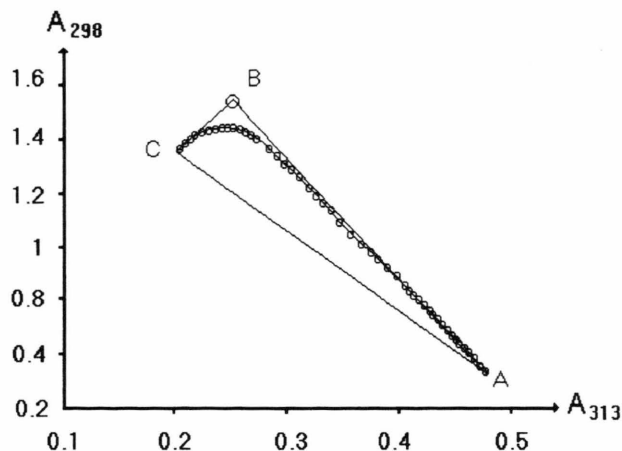
Fig. 2. A diagram A_{313} vs. A_{298} of the photoreduction of anthraquinone-2-carbonic acid in alkaline methanolic solution (absorbance-time data from [19]).

one obtains a negative coordinate in respect of the absorbance at 313 nm in Figure 2. Since negative absorbances have no physical significance, the case $\kappa > 1$ can safely be excluded here (another possibility of distinguishing between $\kappa > 1$ and $\kappa < 1$ is shown in [19, 20] applying the "X-diagram"). From this fact it also follows that the absorption coefficients of the intermediate B can be determined here solely by absorbance data using an A diagram and (b).

2.1.2 Linear Regression Methods

a) The Method of Formal Integration

For the case $s = 2$, (7) leads to ($\lambda = 1, 2$)

$$\dot{A}_1 = z_{10} + z_{11} A_1 + z_{12} A_2,$$
$$\dot{A}_2 = z_{20} + z_{21} A_1 + z_{22} A_2$$

with

$$z_{10} = -z_{11} A_{1\infty} - z_{12} A_{2\infty},$$
$$z_{20} = -z_{21} A_{1\infty} - z_{22} A_{2\infty} \quad \text{and} \quad \dot{A}_\lambda = \frac{dA_\lambda}{d\theta}.$$

The coefficient z_{ij} can be accurately determined by formal integration [1, 2, 4] using linear regression methods. The constants k_1 and k_2 can be calculated from z_{ij} as follows:

$$k_{1,2} = \frac{-S \pm \sqrt{S^2 - 4D}}{2}$$

with $S = z_{11} + z_{22}$ and $D = z_{11} z_{22} - z_{12} z_{21}$.

The subsequent values were found for the measuring example:

$$k_1 = 6.18 \cdot 10^{-4} s^{-1} \quad \text{and} \quad k_2 = 1.01 \cdot 10^{-4} s^{-1}.$$

b) Absorbance Difference Equations of First Order

The photoreduction of anthraquinone-2-carbonic acid can also be evaluated according to the following absorbance difference equations of first order ([2, 4]):

$$\Delta A_1(\theta) = z'_{11} [A_1(\theta) - A_{1\infty}] + z'_{12} [A_2(\theta) - A_{2\infty}],$$
$$\Delta A_2(\theta) = z'_{21} [A_1(\theta) - A_{1\infty}] + z'_{22} [A_2(\theta) - A_{2\infty}]$$

with $\Delta A_\lambda(\theta) = A_\lambda(\theta + \Delta) - A_\lambda(\theta)$

(Δ indicates a constant time difference of the magnitude of the half time of the faster reaction step).
From z'_{ij} it follows for k_1 and k_2

$$k_{1,2} = -\frac{1}{\Delta} \ln \left[\frac{2 + S' \pm \sqrt{S'^2 - 4D'}}{2} \right]$$

with $S' = z'_{11} + z'_{22}$ and $D' = z'_{11} z'_{22} - z'_{12} z'_{21}$.

Applying this algorithm to the measuring example one obtains

$$k_1 = 6.69 \cdot 10^{-4} s^{-1} \quad \text{and} \quad k_2 = 1.13 \cdot 10^{-4} s^{-1}.$$

The partial quantum yields φ_1^A and φ_1^B can be calculated from the equations

$$k_1 = I_0 \varepsilon'_A \varphi_1^A \quad \text{and} \quad k_2 = I_0 \varepsilon'_B \varphi_2^B$$

provided I_0, ε'_A and ε'_B are known. Since ε'_B could be obtained by the point B in Fig. 2, and I_0 was determined by a chemical actinometer ([19]), the final results are

$$\varphi_1^A = 0.10 \quad \text{and} \quad \varphi_2^B = 0.03.$$

The measuring example clearly demonstrates the advantage of the formalism presented here.

3. Theorem 3 on Two Linearly Independent Reactions ($s = 2$)

Thermally controlled reaction systems, i.e. not photoreactions, including reactions of second (and first) order can be developed with a Taylor expansion up to the quadratic term. Reaction systems consisting of s linearly independent degrees of advancement can be described generally by [21, 22]

$$\dot{X} = \dot{X}_0 + \mathbf{K} X + \mathbf{K}'' X^2$$
$$= \mathbf{K}' (X - X_\infty) + \mathbf{K}'' (X - X_\infty)^2. \tag{12}$$

X, X_∞, $\dot{X}$ and $\dot{X}_0$ are column vectors with s elements. X^2 and $(X - X_\infty)^2$ are column vectors with $s(s+1)/2$ elements. The Jacobian matrices $\mathbf{K}$ and $\mathbf{K}'$ are quadratic $s \cdot s$ matrices. $\mathbf{K}''$ is a rectangular matrix with s rows and $s(s+1)/2$ columns.

In the following only non-linear reaction systems with two linearly independent reaction steps ($s = 2$) are considered. At least one reaction step consists of a reaction of second order (the other reaction step can be a reaction of first or second order). Thus, the quantities of (12) are defined as

$$X = \begin{pmatrix} X_1 \\ X_2 \end{pmatrix}, \quad X_\infty = \begin{pmatrix} X_{1\infty} \\ X_{2\infty} \end{pmatrix}, \quad \dot{X} = \begin{pmatrix} \dot{X}_1 \\ \dot{X}_2 \end{pmatrix},$$

$$\dot{X}_0 = \begin{pmatrix} \dot{X}_{10} \\ \dot{X}_{20} \end{pmatrix},$$

$$X^2 = \begin{pmatrix} X_1^2 \\ X_2^2 \\ X_1 X_2 \end{pmatrix},$$

$$(X - X_\infty)^2 = \begin{pmatrix} (X_1 - X_{1\infty})^2 \\ (X_2 - X_{2\infty})^2 \\ (X_1 - X_{1\infty})(X_2 - X_{2\infty}) \end{pmatrix},$$

$$\mathbf{K} = \begin{pmatrix} \partial\dot{X}_1/\partial X_1 & \partial\dot{X}_1/\partial X_2 \\ \partial\dot{X}_2/\partial X_1 & \partial\dot{X}_2/\partial X_2 \end{pmatrix}_{t=0},$$

$$\mathbf{K}' = \begin{pmatrix} \partial\dot{X}_1/\partial X_1 & \partial\dot{X}_1/\partial X_2 \\ \partial\dot{X}_2/\partial X_1 & \partial\dot{X}_2/\partial X_2 \end{pmatrix}_{t\to\infty},$$

$$\mathbf{K}'' = \begin{pmatrix} \partial^2\dot{X}_1/\partial X_1^2 & \partial^2\dot{X}_1/\partial X_2^2 & 2\partial^2\dot{X}_1/\partial X_1\partial X_2 \\ \partial^2\dot{X}_2/\partial X_1^2 & \partial^2\dot{X}_2/\partial X_2^2 & 2\partial^2\dot{X}_2/\partial X_1\partial X_2 \end{pmatrix}/2. \tag{13}$$

The expression for the absorbance corresponding to (12) can be derived from (1) by differentiation with respect to time:

$$\dot{A} = \mathbf{Q}\,\dot{X}. \tag{14}$$

Multiplying (12) with $\mathbf{Q}$, one obtains

$$\dot{A} = \dot{A}_0 + \mathbf{Q}\,\mathbf{K}\,X + \mathbf{Q}\,\mathbf{K}''\,X^2$$
$$= \mathbf{Q}\,\mathbf{K}'(X - X_\infty) + \mathbf{Q}\,\mathbf{K}''(X - X_\infty)^2 \tag{15}$$

with ($s = 2$)

$$\left(\dot{A} = \begin{array}{c} \dot{A}_1 \\ \dot{A}_2 \end{array}\right), \quad \dot{A}_0 = \begin{pmatrix} \dot{A}_{10} \\ \dot{A}_{20} \end{pmatrix}, \quad \mathbf{Q} = \begin{pmatrix} Q_{11} & Q_{12} \\ Q_{21} & Q_{22} \end{pmatrix}.$$

The vectors X^2 and $(X - X_\infty)^2$ can be calculated according to the equations

$$X^2 = \mathbf{P}\,\Delta A^2, \quad (X - X_\infty)^2 = \mathbf{P}(A - A_\infty)^2 \tag{16}$$

with the definitions $(\Delta A_\lambda = A_\lambda - A_{\lambda 0})$

$$\Delta A^2 = \begin{pmatrix} \Delta A_1^2 \\ \Delta A_2^2 \\ \Delta A_1 \Delta A_2 \end{pmatrix},$$

$$(A - A_\infty)^2 = \begin{pmatrix} (A_1 - A_{1\infty})^2 \\ (A_2 - A_{2\infty})^2 \\ (A_1 - A_{1\infty})(A_2 - A_{2\infty}) \end{pmatrix},$$

$$\mathbf{P} = \begin{pmatrix} Q_{22}^2 & Q_{12}^2 & -2Q_{12}Q_{22} \\ Q_{21}^2 & Q_{11}^2 & -2Q_{11}Q_{21} \\ -Q_{21}Q_{22} & -Q_{11}Q_{12} & (Q_{11}Q_{22}+Q_{12}Q_{21}) \end{pmatrix} / |\mathbf{Q}|^2$$

($|\mathbf{Q}|$ is the determinant of $\mathbf{Q}$).

It follows from (15) and (16) [2, 21, 22] that

$$\dot{A} = \dot{A}_0 + \mathbf{Z}\,\Delta A + \mathbf{Y}\,\Delta A^2$$
$$= \mathbf{Z}'(A - A_\infty) + \mathbf{Y}(A - A_\infty)^2 \tag{17}$$

with

$$\Delta A = \begin{pmatrix} A_1 - A_{10} \\ A_2 - A_{20} \end{pmatrix}, \quad (A - A_\infty) = \begin{pmatrix} A_1 - A_{1\infty} \\ A_2 - A_{2\infty} \end{pmatrix}.$$

The matrices $\mathbf{Z}$, $\mathbf{Z}'$, and $\mathbf{Y}$ have the meaning

$$\mathbf{Z} := \mathbf{Q}\,\mathbf{K}\,\mathbf{Q}^{-1}, \quad \mathbf{Z}' := \mathbf{Q}\,\mathbf{K}'\,\mathbf{Q}^{-1} \quad \text{and}$$
$$\mathbf{Y} := \mathbf{Q}\,\mathbf{K}''\,\mathbf{P}.$$

The matrix $\mathbf{Q}$ transforms the two degrees of advancement X_1 and X_2 into the absorbance differences $(A_1 - A_{10})$ and $(A_2 - A_{20})$ according to (1). The indices 1 and 2 refer to the two different wavelengths measured. These wavelengths must be chosen in such a way that $\mathbf{Q}$ is regular (this information may be taken from the absorbance or absorbance difference quotient diagrams as shown in [1, 2, 13, 14]).

The matrices $\mathbf{K}$, $\mathbf{K}'$, and $\mathbf{K}''$ characterize the individual reaction systems. However, only the elements of the transformed matrices $\mathbf{Z}$, $\mathbf{Z}'$, and $\mathbf{Y}$ can be obtained from spectroscopic measurements according to (17). The constants z_{ij} and y_{ij} or z'_{ij} and y_{ij} can be determined by formal integration when the absorbances A_1 and A_2 are measured as functions of time.

$\mathbf{Z}$ and $\mathbf{K}$ are similar matrices as are $\mathbf{Z}'$ and $\mathbf{K}'$. $\mathbf{Z}$ and $\mathbf{K}$ have the same trace and determinant and, therefore, the same eigenvalues. The same relationship exists between $\mathbf{Z}'$ and $\mathbf{K}'$. $\mathbf{Y}$ and $\mathbf{K}''$ are equivalent matrices. In general, the following rules hold [21]:

– $\mathbf{K}$ (and $\mathbf{Z}$, respectively) must have at least the rank one.

– $\mathbf{K}'$ (and $\mathbf{Z}'$, respectively) can have the rank zero when $\mathbf{K}''$ (and $\mathbf{Y}$, respectively) has the rank two.
– $\mathbf{K}''$ (and $\mathbf{Y}$) has at least the rank one (when the rank of $\mathbf{K}''$ is zero, no reaction of second order is involved in the mechanism, which is in opposition to the assumed system treated here).

3.1. Theoretical Examples

3.1.1 The Mechanism $2\,\mathrm{A}\xrightarrow{k_1}\mathrm{B}\xrightarrow{k_2}\mathrm{C}$

To this mechanism the following matrices belong

$$\mathbf{K} = \begin{pmatrix} -4\,k_1\,a_0 & 0 \\ k_2 & -k_2 \end{pmatrix}, \quad \mathbf{K}' = \begin{pmatrix} 0 & 0 \\ k_2 & -k_2 \end{pmatrix}$$

and $\quad \mathbf{K}'' = \begin{pmatrix} 4\,k_1 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$

according to (13). The matrices $\mathbf{Z}$ and $\mathbf{K}$ are similar matrices $(\mathbf{Z} = \mathbf{Q}\,\mathbf{K}\,\mathbf{Q}^{-1})$. Therefore, ($S$ = trace and D = determinant of $\mathbf{Z}$)

$$S = z_{11} + z_{22} = 4\,k_1\,a_0 - k_2 \quad \text{and}$$

$$D = z_{11}\,z_{22} - z_{12}\,z_{21} = 4\,k_1\,k_2\,a_0.$$

All coefficients $(z_{ij}, z'_{ij}, y_{ij})$ from (17) can be determined by linear regression, applying formal integration (if necessary in combination with the method of "singular value decomposition" (SVD) as demonstrated in [22]). By varying a_0 the quantities k_1 and k_2 can be determined from the slope and ordinate intercept of the straight line obtained by the plot S vs. a_0. The diagram D vs. a_0 leads to a straight line passing through the origin. The slope here has to agree with the product of slope and ordinate intercept according to the plot S vs. a_0. This procedure, which has proved to be reliable in practice, clearly demonstrates the advantages of (17) for evaluating spectroscopic data.

Results of formal integration can be directly correlated to absorbance diagrams in many cases. In Fig. 3 a typical curve for the mechanism considered is shown in an A diagram. The reaction starts at point A and ends in C. The slope of the tangent to point A corresponds to y_{ij}, for example. It follows from $\mathbf{Y} = \mathbf{Q}\,\mathbf{K}''\,\mathbf{P}$, considering ratios of y_{ij},

$$\frac{y_{11}}{y_{21}} = \frac{Q_{11}}{Q_{21}} = \frac{\varepsilon_{1B} - 2\,\varepsilon_{1A}}{\varepsilon_{2B} - 2\,\varepsilon_{2A}},$$

$$\frac{y_{11}}{y_{12}} = \frac{y_{21}}{y_{22}} = \left(\frac{Q_{22}}{Q_{12}}\right)^2 = \left(\frac{\varepsilon_{2C} - \varepsilon_{2B}}{\varepsilon_{1C} - \varepsilon_{1B}}\right)^2$$
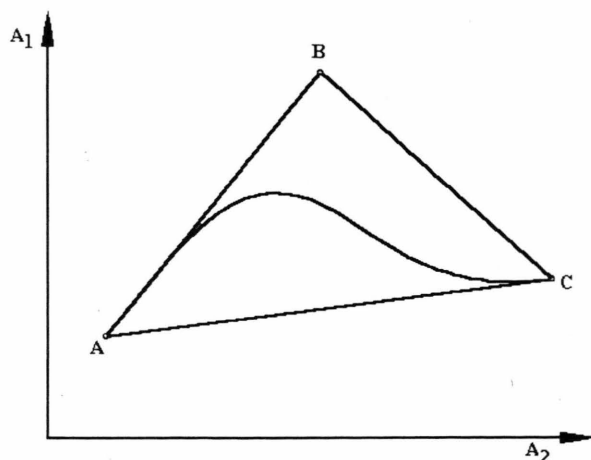
($\mathbf{Y}$ has here the rank one).

Fig. 3. Schematic A diagram for the mechanism $2\,\mathrm{A}\to\mathrm{B}\to\mathrm{C}$.

Since $\dot{A}_0 = Q\,\dot{X}_0$ and

$$\dot{X}_0 = \begin{pmatrix} k_1\,a_0^2 \\ 0 \end{pmatrix},$$

the slope of the tangent at point A in Fig. 3 has the value

$$\left.\frac{\mathrm{d}A_1}{\mathrm{d}A_2}\right|_{t=0} = \frac{Q_{11}}{Q_{21}}.$$

Thus, the ratio y_{11}/y_{21}, which results from the individual y_{ij} obtained by formal integration, indicates the slope of the tangent at point A in Figure 3. Additionally, the ratio $\sqrt{y_{11}/y_{12}}$ supplies the reciprocal slope of the straight line BC in Figure 3. With the slope a straight line can be constructed passing through the point C. The point of intersection resulting from BC and AB leads to point B with the coordinates

$$A_{\lambda B} = \ell\,\varepsilon_{\lambda B}\,\frac{a_0}{2}.$$

3.1.2. The Mechanism $\mathrm{A} + \mathrm{B}\xrightarrow{k_1}\mathrm{C}\xrightarrow{k_2}\mathrm{D}$

To this mechanism the matrix belongs

$$\mathbf{K} = \begin{pmatrix} -k_1\,(a_0 + b_0) & 0 \\ k_2 & -k_2 \end{pmatrix}$$

(with rank two). All two rate constants k_1 and k_2 can be determined when the coefficients of $\mathbf{Z}$ are known (for example by formal integration). The following

equation are true for the trace S and the determinant D of Z:

$$S = z_{11} + z_{22} = -k_1(a_0 + b_0) - k_2,$$
$$D = z_{11}z_{22} - z_{12}z_{21} = k_1 k_2 (a_0 + b_0).$$

By varying a_0 and b_0, the quantities k_1 and k_2 can be obtained from the plot of S vs. $(a_0 + b_0)$, as described in Section 3.1.1.
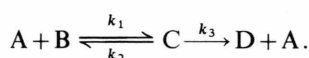
Furthermore, the following equations are true

$$\frac{y_{11}}{y_{21}} = \frac{y_{12}}{y_{22}} = \frac{y_{13}}{y_{23}} = \frac{Q_{11}}{Q_{21}}, \quad \frac{y_{11}}{y_{12}} = \frac{y_{21}}{y_{22}} = \left(\frac{Q_{22}}{Q_{12}}\right)^2,$$

$$\frac{y_{11}}{y_{13}} = \frac{y_{21}}{y_{23}} = -\frac{Q_{22}}{2Q_{12}}, \quad \frac{y_{12}}{y_{13}} = \frac{y_{22}}{y_{23}} = -\frac{Q_{12}}{2Q_{22}}.$$

3.1.3 The Mechanism $A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_3} D + A$

The enzyme mechanism according to Michaelis-Menten is

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_3} D + A.$$

The following equations hold for the trace S and determinant D of Z, provided that Bodenstein's hypothesis is *not* applied on intermediate C:
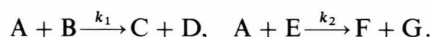
$$S = z_{11} + z_{12} = -k_1(a_0 + b_0) - k_2 - k_3,$$
$$D = z_{11}z_{22} - z_{12}z_{21} = k_1 k_2 a_0.$$

All quantities k_1, k_2, and k_3 are again determinable by the plots S vs. $(a_0 + b_0)$ and D vs. a_0.

About 100 reaction systems having the rank $s = 2$ were studied under the aspects presented here.

A practical example for the application of the theory described here is shown in [22] investigating the mechanism

$$A + B \xrightarrow{k_1} C + D, \quad A + E \xrightarrow{k_2} F + G.$$

3.2. Generalization

Systems with reactions of the second order can be distinguished from each other in many cases. This is in contrast to the distinction of systems consisting only of reaction of the first order and having the same rank in relation to the Jacobian matrices. Thus it is not possible to distinguish spectroscopically between the two systems $A \rightarrow B \rightarrow C$ and $A \rightarrow B$, $C \rightarrow D$. In contrast, it is not a problem to distinguish between the mechanisms $2A \rightarrow B \rightarrow C$, $A + B \rightarrow C \rightarrow D$ and

$A + B \rightleftharpoons C \rightarrow D + A$, as demonstrated above. However, other reaction mechanisms exist which have the same functional dependence as these examples. For example, the reaction system $2A \rightarrow B \rightarrow C$ can not be distinguished spectroscopically from the system $2A \rightarrow B \rightleftharpoons C$. In general, two criteria can be derived for the distinction of two reaction systems [21]. A distinction is not possible

- if the eigenvalues have the same dependence on the initial concentrations,
- if a linear transformation exists which transforms all three matrices K, K', and K'' into each other.

Here, it is meaningful whether the individual elements of the Jacobian matrices K and K' belonging each to the two reaction systems depend solely on k_i or on the initial concentrations, in addition. If there is correspondence, the two reaction systems can not be spectroscopically distinguished from each other. In the other case, the reaction systems can be distinghished by varying the initial concentrations and plotting the corresponding graphs (as for example S vs. a_0 or D vs. a_0).

These correlations lead to

Theorem 3:

Thermal reaction systems which consist of two linearly independent reaction steps – one step of which is at least a reaction of second order – can not be distinguished from each other by purely spectroscopic means if their eigenvalues have the same functional dependence on the initial concentrations.

Another formulation is

Thermal reaction systems with two linearly independent reaction steps are not purely spectroscopically distinguishable from each other when the traces and determinants have the same functional dependence on the initial concentrations.

Both linearly independent reaction steps can be reactions of second order. It is also admitted that *one* linearly independent reaction step consists only of reactions of first order. The eigenvalues mentioned in theorem 3 refer to the matrices K and K'. This is also true for the traces and determinants.

The ability to identify and distinguish between general reaction systems is studied in [23] using another mathematical algorithm as presented here. Several equations are developed there for solving the problem of identification.

Theorem 3 can be generalized since (12) is true for s linearly independent reactions, one reaction step of which is at least of second order (the other steps can be of first order). Equation (17) can be modified for systems consisting of s reaction steps. However, the spectroscopic kinetic analysis of systems with reactions of second order is presumably limited to systems having the rank $s = 2$. The number of z_{ij} to be determined significantly increases largely in the case of $s > 2$. Nevertheless, theorem 3 can be extended to systems with rank s (consisting only of reactions of first and second order or only of reactions of second order).

Thermal reaction systems which consist of s linearly independent reaction steps – one step of which is at least a reaction of second order – can not be distinghuished from each other by purely spectroscopic means if their eigenvalues have the same functional dependence on the initial concentrations.

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