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Determination of kinetic parameters from chemical relaxation data: discrimination between coupled two-step binding reactions differing in stoichiometry

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Abstract. The chemical relaxation times of two different two-step equilibrium reactions, characterized by a 1:1 binding process followed by a subsequent rearrangement step and a stepwise 1:2 binding reaction, are analyzed for the purpose of qualitative model discrimination and quantitative determination of kinetic parameters. The equations describing the dependences of the two reciprocal relaxation times on suitable concentrations are given for both models in the general case as well as for four different limiting situations which are characterized by well separated relaxation times. The conditions corresponding to the limiting cases are expressed in terms of strong, weak and no coupling between the two partial equilibrium steps involved in both models. The coupling strength depends on the rate constants as well as on the total concentrations of the reactants. Criteria to discriminate between these two reaction models under defined limiting conditions are developed. In the general case, the product of both reciprocal relaxation times can be used to distinguish both models. If only one relaxation time can be resolved experimentally, it is possible under conditions described to determine only a reduced set of individual rate constants for most of the limiting cases considered. If both relaxation times are observed, all rate constants are determinable in the general case as well as in most of the limiting cases discussed.

Key words: Chemical relaxation – Relaxation kinetics – Two-step binding reactions – Relaxation times – Kinetic parameters

Introduction

Chemical relaxation spectrometry (Eigen and De Maeyer 1974) is widely used for elucidating the mechanism of a given chemical or biochemical equilibrium reaction and for determining the corresponding rate constants. In this method the thermodynamic equilibrium of the chemical system investigated is disturbed by quickly changing a suitable external parameter, such as temperature or pressure, for example. The subsequent equilibration of the system occurs in the form of concentration changes which can be followed by employing a time-resolved detection of an appropriate parameter, e.g. the absorption of the solution at a certain wavelength, provided perturbation and detection are fast enough. If the concentration changes induced by the perturbation are small compared to the corresponding equilibrium concentrations, the equilibration process is described in the case of transient methods by a sum of decaying exponentials, each being characterized by an individual positive relaxation time and a specific positive or negative amplitude. The number of resolvable exponentials is equal to or less than the number of equilibrium steps.

In general, the relaxation times depend on the rate constants of all individual reaction steps and on equilibrium concentrations of the species involved, but not on the type, direction or magnitude of the disturbance, as long as the induced concentration changes are small enough. Therefore, it is not possible in general to associate a particular relaxation time with an individual reaction step. However, if simplifying assumptions can be made, a particular relaxation time depends under certain conditions only on the rate constants of a single reaction step, although stability constants of fast pre-equilibria may frequently be involved, too.

In order to postulate a particular reaction model on the basis of chemical relaxation data, simple experimental criteria, such as the number of observed relaxation times, are of importance. If, for instance, two relaxation times are determined experimentally, every mechanism involving only a single reaction step can be excluded. The inverse conclusion may be wrong: Observing only one relaxation process does not exclude two-step mechanisms, because the other process may proceed too fast or too slow for detection, or may have an amplitude which is too

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low to be resolved from the data. Another criterion is provided by the relationship between the relaxation times on the one hand and the rate constants and equilibrium concentrations and constants on the other hand, which is expected for a pre-chosen reaction model. Thus, a reaction mechanism cannot be proven by applying chemical relaxation methods, but a detailed characterization of a postulated model is possible as long as it cannot be ruled out by the available experimental data.

Many chemical or biochemical equilibrium reactions are characterized by one of the following, comparatively simple models:

Model 1:

$$A+B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C \qquad K_{12}=\frac{k_{12}}{k_{21}} \tag{1}$$

Model 2:

$$A+B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} D \qquad K_{12}=\frac{k_{12}}{k_{21}} \quad K_{23}=\frac{k_{23}}{k_{32}} \tag{2}$$

Model 3:

$$A+2B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} C+B \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} D \quad K_{12}=\frac{k_{12}}{k_{21}} \quad K_{23}=\frac{k_{23}}{k_{32}} \tag{3}$$

The stability constants K_{ij} ($i=1$ or $2, j=i+1$) are specified by the quotients of the corresponding rate constants k_{ij}/k_{ji}. These reaction schemes include the case where state C and/or D is formed via one or more intermediate states (not shown in (1), (2) and (3)) being present at comparatively low concentrations (steady states); the experimentally accessible overall rate constants are then functions of the rate constants related to the intermediate states involved (Eigen and De Maeyer 1974). Equation (1) describes, for instance, the most simple complex formation reaction exhibiting 1:1 stoichiometry. It is characterized by a single relaxation time, τ_1. If, however, the binding between, for example, a molecule or ion B and the ligand A, leading to the complex C, is followed by a subsequent rearrangement to state D, the two-step model 2 applies, which is characterized by two relaxation times, τ_1 and τ_2. Another two-step mechanism is given by (3). Instead of the first-order rearrangement reaction assumed in (2), a second molecule of B is bound to the initially formed complex C leading to the final reaction state D, being characterized by a 1:2 stoichiometry. Owing to their general importance, both two-step binding models have often to be considered for the analysis of kinetic data obtained from biochemical studies, especially in enzyme reactions.

In principle, chemical relaxation data can be utilized to distinguish models 1, 2 and 3. Models 1 and 2 are dealt with comprehensively in many review articles or text books concerning chemical relaxation (e.g. by Eigen and De Maeyer 1974; Czerlinsky 1966; Strehlow and Knoche 1977). On the other hand, model 3 is considered by Czerlinsky only under the assumption that one of the reaction steps equilibrates rapidly compared to the other one. A rough comparison of model 2 and 3 may suggest that it should be fairly easy to differentiate between them. However, as will be seen later, this is not possible under all conditions in a straightforward manner. Even under simple conditions when the second equilibrium in model 2 and 3 adjusts much slower than the corresponding initial binding step, it can be difficult to distinguish between the models within certain concentration ranges on the basis of the usual concentration dependence of the reciprocal relaxation times. Under the same condition the concentration dependences allow one also to determine the rate constants by comparatively simple procedures. But if only experimental conditions are achievable where the relaxation times τ_1 and τ_2 are similar, the corresponding expressions characterizing models 2 and 3 are rather complex prohibiting those simple methods, but model discrimination as well as the determination of the rate constants is, of course, also desirable under these circumstances. For the latter purpose, a plot procedure has been suggested for model 2 (Havsteen 1967). Model 3 and thus also its differentiation from 2 under such conditions has not been examined up to now.

The aim of this article is to find conditions that allow one to distinguish between the reaction models 2 and 3 on the basis of the expected concentration dependences of the corresponding reciprocal relaxation times. For this purpose, the general expressions as well as the cases of strongly coupled, of weakly coupled and completely uncoupled equilibria under different approximative conditions are considered and analyzed. The corresponding equations for the relaxation times will be given. A set of characteristic rate constants is applied to calculate the concentration dependences of the reciprocal relaxation times for models 2 and 3. This illustration is expected to provide helpful hints for future experimental studies concerning the evaluation of reaction mechanisms. No relaxation amplitude considerations are included within the scope of this article although they are believed to be relevant, too, for the discrimination between mechanisms.

Dependences of the relaxation times on equilibrium concentrations

General expressions

The principles of calculation of the dependence of reciprocal relaxation times $1/\tau_i$ (here: $i=1$ or 2) on equilibrium concentrations $\underline{c}_l$ of involved species (identified by their index; here: $l=A$, B, C or D) for any assumed reaction model are given in detail in the literature (e.g. Eigen and De Maeyer 1974; Strehlow and Knoche 1977). In the case of the one-step binding equilibrium of model 1 the single relaxation time τ_1 (Eigen and De Maeyer 1974) is determined by:

$$\frac{1}{\tau_1}=k_{21}+k_{12}(\underline{c}_A+\underline{c}_B) \tag{4}$$

while in the case of any two-step reaction the two predicted relaxation times τ_1 and τ_2 are given by (Eigen and De Maeyer 1974):

$$\frac{1}{\tau_{1,2}}=\frac{\alpha}{2}(1\pm\sqrt{1-\beta}) \quad \alpha\equiv-a_{11}-a_{12}$$

$$\beta\equiv 4\,\frac{a_{11}a_{22}-a_{12}a_{21}}{(a_{11}+a_{22})^2}\,. \tag{5}$$

The positive sign of the square root term leads to the expression for τ_1, the negative sign to that for τ_2. The coefficients α and β must be positive and β cannot exceed unity. Otherwise, at least one of the relaxation times would be negative or complex, which is meaningless. Thus, according to (5) τ_1 is always smaller than τ_2. Each individual type of two-step reaction is characterized by its dependence of the coefficients a_{ij} ($i, j = 1$ or 2) on rate constants and equilibrium concentrations. These concentrations are related to the total concentration of the reactants by the mass balance equations. For models 2 and 3 the total reactant concentrations c_{A0} and c_{B0}, respectively, are given by:

$$c_{A0} = \underline{c}_A + \underline{c}_C + \underline{c}_D \qquad c_{B0} = \underline{c}_B + \underline{c}_C + f\,\underline{c}_D \tag{6}$$

with $f = 1$ for model 2 and $f = 2$ for model 3. The equilibrium concentrations can be calculated from these mass balance equations by the corresponding equilibrium constants (Czerlinsky 1966; Bremer and Grell 1994). In the case of model 2 the coefficients a_{ij} are given by (Czerlinsky 1966):

$$\begin{aligned} a_{11} &= -k_{21} - k_{12}(\underline{c}_A + \underline{c}_B) \qquad & a_{12} &= -k_{21} \\ a_{21} &= -k_{23} & a_{22} &= -k_{23} - k_{32} \end{aligned} \tag{7}$$

and for model 3 (Bremer 1992) by:

$$\begin{aligned} a_{11} &= -k_{21} - k_{12}(\underline{c}_A + \underline{c}_B) \qquad & a_{12} &= -k_{21} + k_{12}\underline{c}_A \\ a_{21} &= k_{23}(\underline{c}_C - \underline{c}_B) & a_{22} &= -k_{32} - k_{23}(\underline{c}_C + \underline{c}_B) \end{aligned} \tag{8}$$

These coefficients result from the system of linearized differential equations for the time course of the deviations $x_s = c_s - \underline{c}_s$ ($s = A$ or D) of the actual concentrations c_s from their equilibrium values $\underline{c}_s$:

$$\frac{\mathrm{d}x_A}{\mathrm{d}t} = a_{11}x_A + a_{12}x_D \qquad \frac{\mathrm{d}x_D}{\mathrm{d}t} = a_{21}x_A + a_{22}x_D \tag{9}$$

using the mass balance equations (6) and assuming constant equilibrium concentrations. Formulating the differential equations for other species affects only the expressions for a_{ij}; the general dependencies of the relaxation times on the equilibrium concentrations and rate constants are not influenced. The coefficients describe the influence of the concentration changes of both species on their time course. Thus, the strength of coupling between both equilibria involved in the models 2 and 3 depends on the absolute values of the different coefficients a_{ij} and hence on the rate constants as well as via the equilibrium concentrations of the involved species also on the total concentrations of the reactants.

Concerning the general case of any two-step mechanism, the dependence of the reciprocal relaxation times on the coefficients a_{ij} (5) and hence also on the involved concentrations is rather complicated as a consequence of the structure of the coefficient β as well as of the occurrence of the square root. Thus, a straightforward evaluation of single reciprocal relaxation times in order to obtain the individual rate constants is not possible as in the case of model 1 where a plot of $1/\tau_1$ versus $(\underline{c}_A + \underline{c}_B)$ yields a straight line with the slope k_{12} and the intercept of the ordinate k_{21}. However, according to (5) the sum and the product of both reciprocal relaxation times for two-step reactions result both in quite simple expressions in terms of the coefficients a_{ij} and thus also of the concentrations:

$$\begin{aligned} &\frac{1}{\tau_1} + \frac{1}{\tau_2} = \alpha = -a_{11} - a_{22} \\ &\frac{1}{\tau_1}\frac{1}{\tau_2} = \frac{\alpha^2\beta}{4} = a_{11}a_{22} - a_{12}a_{21}. \end{aligned} \tag{10}$$

For model 2, both expressions lead to linear functions of $(\underline{c}_A + \underline{c}_B)$ according to (7):

$$\frac{1}{\tau_1} + \frac{1}{\tau_2} = k_{21} + k_{23} + k_{32} + k_{12}(\underline{c}_A + \underline{c}_B) \tag{11a}$$

$$\frac{1}{\tau_1}\frac{1}{\tau_2} = k_{21}k_{32} + k_{12}(k_{23} + k_{32})(\underline{c}_A + \underline{c}_B). \tag{11b}$$

As indicated earlier (Havsteen 1967), plotting the sum and the product of the reciprocal relaxation times versus $(\underline{c}_A + \underline{c}_B)$ yields straight lines. All four rate constants can then be calculated from the four different coefficients determining these lines. The corresponding expressions for model 3 are not as simple as in the case of model 2. According to (8) two different concentration terms contribute to the sum of both reciprocal relaxation times and the product contains also products of equilibrium concentrations:

$$\frac{1}{\tau_1} + \frac{1}{\tau_2} = k_{21} + k_{32} + k_{12}(\underline{c}_A + \underline{c}_B) + k_{23}(\underline{c}_C + \underline{c}_B) \tag{12a}$$

$$\begin{aligned} \frac{1}{\tau_1}\frac{1}{\tau_2} &= k_{21}k_{32} + k_{12}k_{32}(\underline{c}_A + \underline{c}_B) \\ &\quad + k_{12}k_{23}(4\underline{c}_A + \underline{c}_B + \underline{c}_C)\underline{c}_B \end{aligned} \tag{12b}$$

Hence, no simple determination of the rate constants is possible in a way similar to that for model 2.

Approximations

In order to notice easily recognizable differences between the two models 2 and 3, the consideration of limiting cases is a helpful approach. In the following, four different limiting cases are discussed. Each case is characterized by two experimentally resolved, well separated (at least by a factor of 10) relaxation times. The approximate relaxation times corresponding to the limiting cases are denoted by $\tilde{\tau}_i$ in order to discriminate them from the exact relaxation times τ_i. As will be shown below the previous condition $\tau_1 < \tau_2$ no longer holds for all approximate expressions and all concentrations. Thus, without any preknowlewdge the experimentally determined relaxation times cannot immediately be assigned to either $\tilde{\tau}_1$ or $\tilde{\tau}_2$. Hence, the experimentalist must perform this assignment according to the theoretical concentration dependences of the reciprocal relaxation times assuming a particular reaction model in a given concentration range (for details see below). For the following it is assumed that the problem of assignment could be solved.

If one relaxation time is small compared to the other one, this is according to (5) equivalent to the condition

Table 1. Expressions for the approximate reciprocal relaxation times $1/\tilde{\tau}_i$ according to the two-step reaction models 2 and 3 for different conditions, provided that the relaxation times are well separated. Case I): strong coupling between both partial equilibria; case II): the first reaction step equilibrates much faster than the second one (special case of weak coupling); case III): the first reaction step adjusts very slowly compared to the second one (special case of weak coupling); case IV): no coupling between the equilibria

Case	Model	$1/\tilde{\tau}_1$	$1/\tilde{\tau}_2$
I)	2	$k_{21}+k_{23}+k_{32}+k_{12}(\underline{c}_A+\underline{c}_B)$	$\frac{k_{21}\,k_{32}+k_{12}(k_{23}+k_{32})(\underline{c}_A+\underline{c}_B)}{k_{21}+k_{23}+k_{32}+k_{12}(\underline{c}_A+\underline{c}_B)}$
	3	$k_{21}+k_{32}+k_{12}(\underline{c}_A+\underline{c}_B)+k_{23}(\underline{c}_C+\underline{c}_B)$	$\frac{k_{21}\,k_{32}+k_{12}\,k_{32}(\underline{c}_A+\underline{c}_B)+k_{12}\,k_{23}\,\underline{c}_B(4\underline{c}_A+\underline{c}_B+\underline{c}_C)}{k_{21}+k_{32}+k_{12}(\underline{c}_A+\underline{c}_B)+k_{23}(\underline{c}_C+\underline{c}_B)}$
II)	2	$k_{21}+k_{12}(\underline{c}_A+\underline{c}_B)$	$k_{32}+k_{23}\frac{\underline{c}_A+\underline{c}_B}{\underline{c}_A+\underline{c}_B+K_{12}^{-1}}$
	3	$k_{21}+k_{12}(\underline{c}_A+\underline{c}_B)$	$k_{32}+k_{23}\frac{\underline{c}_B(4\underline{c}_A+\underline{c}_B+\underline{c}_C)}{\underline{c}_A+\underline{c}_B+K_{12}^{-1}}$
III)	2	$k_{23}+k_{32}$	$k_{21}\frac{1}{1+K_{23}}+k_{12}(\underline{c}_A+\underline{c}_B)$
	3	$k_{32}+k_{23}(\underline{c}_C+\underline{c}_B)$	$k_{12}\left(\underline{c}_A\frac{2\underline{c}_B+K_{23}^{-1}}{\underline{c}_C+\underline{c}_B+K_{23}^{-1}}+\underline{c}_B\right)+k_{21}\frac{2\underline{c}_C+K_{23}^{-1}}{\underline{c}_C+\underline{c}_B+K_{23}^{-1}}$
IV)	2	$k_{21}+k_{12}(\underline{c}_A+\underline{c}_B)$	$k_{23}+k_{32}$
3	3	$k_{21}+k_{12}(\underline{c}_A+\underline{c}_B)$	$k_{32}+k_{23}(\underline{c}_C+\underline{c}_B)$

$\beta \ll 1$, while the condition $\tau_1 \approx \tau_2$ is equivalent to $\beta \approx 1$. Thus, in the case of two well separated relaxation times the general expression (5) can be approximated by:

$$\frac{1}{\tau_1} \approx \frac{1}{\tilde{\tau}_1} \equiv -a_{11}-a_{22} \qquad \frac{1}{\tau_2} \approx \frac{1}{\tilde{\tau}_2} \equiv \frac{a_{12}a_{21}-a_{11}a_{22}}{a_{11}+a_{22}} \tag{13}$$

These equations include all those limiting cases which are discussed in the following. The first case assumes that both equilibria of models 2 and 3 are strongly coupled (I). Secondly, two cases of weak coupling between the equilibria are considered (II, III). For models 2 and 3 this is identical to the assumption that one reaction step proceeds very fast compared to the other one. Finally, the completely uncoupled case (IV) is discussed. The conditions which characterize these limiting cases depend on the numerical values of the coefficients a_{ij} and hence on the rate constants as well as on the total concentrations of the reactants. The resulting theoretical equations describing the dependences of the approximate reciprocal relaxation times on equilibrium concentrations under different conditions are derived and compiled in Table 1. The chosen examples, which will be shown graphically in Fig. 3, provide a comparison of some of these approximations with the dependences calculated by employing the exact equations.

I) If $|a_{11}|$ is comparable to $|a_{12}|$ as well as $|a_{21}| \approx |a_{22}|$ or if in terms of the matrix formalism (Eigen and De Maeyer 1974) all matrix elements within one row are comparable to each other, then both equilibria are strongly coupled: The concentration changes of $A(x_A)$ and $D(x_D)$ markedly contribute to equations (9). Under these circumstances β is small compared to 1 and hence both relaxation times are well separated. The corresponding expressions of the formula describing the approximate reciprocal relaxation times $1/\tilde{\tau}_i$ for models 2 and 3 are given in Table 1.

II) If $|a_{11}|$ is large compared to $|a_{21}|$ as well as to $|a_{22}|$ (i.e. the first diagonal element of the coefficient matrix dominates over all elements of the second row), then the first reaction step of model 2 or 3 adjusts rapidly compared to the second one (here: both $k_{12}(\underline{c}_A+\underline{c}_B)$ and k_{21} are large compared to either k_{23} and k_{32} in the case of model 2 or to $k_{23}(\underline{c}_C+\underline{c}_B)$ and k_{32} in the case of model 3. For both models, again β is small compared to 1, because $|a_{12}|$ cannot exceed $|a_{11}|$ since this would require either a negative concentration or a negative stability constant. The corresponding equations for the reciprocal relaxation times of this weakly coupled case can be deduced from Table 1. For model 2 they are given in textbooks (Eigen and De Maeyer 1974; Czerlinsky 1966); for model 3, however, only for $1/\tilde{\tau}_1$ (Czerlinsky 1966).

III) In a similar way to II), if $|a_{22}| \gg |a_{11}|$, $|a_{12}|$ (the second diagonal element of the coefficient matrix dominates over all elements of the first row), then the second reaction step in model 2 or 3 equilibrates much faster than the first one. For both models, again the relation $\beta \ll 1$ holds, because it is not possible that $|a_{21}| > |a_{22}|$. The related equations are specified in Table 1. They are also dealt with in a textbook (Czerlinsky 1966).

IV) Finally, if $|a_{11}| \gg |a_{12}|$ and $|a_{22}| \gg |a_{21}|$ (the main diagonal elements dominate over the remaining elements of the corresponding row), then both equilibria in model 2 or 3 are completely uncoupled: The concentration of species D, for example, has no influence on the formation rate of species A and vice-versa. Thus, the system of differential equations splits into two independent equations, each describing only a single equilibrium of the reaction scheme. Only in this case is a straightforward assignment of both measured relaxation times to the individual reaction step possible. If the diagonal elements are of the same size, then both relaxation times are of similar magnitude ($\beta \approx 1$); if one of the diagonal elements is large compared to the other one, then the relaxation times are well separated ($\beta \ll 1$). The expressions determining the approximate reciprocal relaxation times are also given in Table 1. They are identical to those found in the literature (Eigen and De Maeyer 1974; Czerlinsky 1966; Strehlow and Knoche 1977) assuming both reaction steps to be isolated reactions.

The discussion of these four limiting cases implies that whether or not a particular relaxation time is large compared to the other one does not allow one to make any a priori statement about the degree of coupling between both equilibria involved in the reaction, nor about the equilibration time of one individual reaction step relative to that of the other one. If, for instance, both individual reaction steps adjust within the same time (in terms of the matrix elements expressed as $a_{11}=a_{22}$) but both equilibria are strongly coupled (case I), then according to (13) the ratio of the experimentally accessible relaxation times τ_1/τ_2 is small compared to unity.

If c_{A0} is very small compared to c_{B0}, different types of approximations than in the case of well separated relaxation times can be considered. Since the inequalities $\underline{c}_A$, $\underline{c}_C$, $\underline{c}_D \leq c_{A0}$ and $\underline{c}_B \leq c_{B0}$ hold under any condition because of the mass balance equations (6), $\underline{c}_B$ is approximately equal to c_{B0} in the case $c_{A0} \ll c_{B0}$. Thus, the equations describing the coefficients a_{ij} (7) and (8) and, accordingly, the reciprocal relaxation times under all conditions considered above can be simplified significantly. Especially useful is this approximation for the evaluation of the sum and the product of the reciprocal relaxation times. In the case of model 2 linear dependences on c_{B0} are obtained:

$$\frac{1}{\tau_1}+\frac{1}{\tau_2} \approx k_{21}+k_{32}+k_{23}+k_{12}\,c_{B0} \tag{14a}$$

$$\frac{1}{\tau_1}\,\frac{1}{\tau_2} \approx k_{21}\,k_{32}+k_{12}\,(k_{23}+k_{32})\,c_{B0}\,. \tag{14b}$$

In the case of model 3 the corresponding expressions are:

$$\frac{1}{\tau_1}+\frac{1}{\tau_2} \approx k_{21}+k_{32}+(k_{12}+k_{23})\,c_{B0} \tag{15a}$$

$$\frac{1}{\tau_1}\,\frac{1}{\tau_2} \approx k_{21}\,k_{32}+k_{12}\,k_{32}\,c_{B0}+k_{12}\,k_{23}\,c_{B0}^2\,. \tag{15b}$$

Thus, for $c_{A0} \ll c_{B0}$ the sum of the reciprocal relaxation times is a linear function of c_{B0} for both models considered here, while the product depends quadratically on this concentration parameter only in the case of model 3.

Discussion

Analysis of the general expressions

In order to discuss the general expressions (5) describing the dependence of the reciprocal relaxation times on concentrations it is important to check for relative extrema of these functions. For this purpose the first and second derivatives with respect to suitable concentration variables are derived and analyzed for the models 2 and 3. Owing to the characteristic concentration dependences of the coefficients a_{ij} of a particular model no general expressions of these derivatives, which are valid for every two-step model, can be obtained.

Model 2:

In the case of model 2 the first derivative of the reciprocal relaxation times with respect to the sum of the free reactant concentrations is given by (the upper sign corresponds to τ_1, the lower to τ_2):

$$\frac{d\,1/\tau_{1,2}}{d(\underline{c}_A+\underline{c}_B)} = \frac{k_{12}}{2} \cdot \left\{1 \mp \frac{k_{23}+k_{32}-k_{12}(\underline{c}_A+\underline{c}_B)-k_{21}}{\sqrt{[k_{23}+k_{32}-k_{12}(\underline{c}_A+\underline{c}_B)-k_{21}]^2+4k_{21}k_{23}}}\right\} \tag{16}$$

Since $k_{21}\,k_{23} \geq 0$, the quotient term given in the curly brackets cannot exceed unity. Hence, both derivatives are characterized by positive values only. Therefore, no relative minimum or maximum of the reciprocal relaxation times as function of $(\underline{c}_A+\underline{c}_B)$ can occur: $1/\tau_{1,2}$ is strictly monotonically increasing with increasing concentrations. The second derivative provides additional information about this function (again, the upper sign corresponds to τ_1, the lower to τ_2):

$$\frac{d^2\,1/\tau_{1,2}}{d(\underline{c}_A+\underline{c}_B)^2} = \pm\frac{2k_{12}^2\,k_{21}\,k_{23}}{\{[k_{23}+k_{32}-k_{12}(\underline{c}_A+\underline{c}_B)-k_{21}]^2+4k_{21}\,k_{23}\}^{3/2}} \tag{17}$$

From this expression it is concluded that $1/\tau_1$ always has a positive curvature and $1/\tau_2$ a negative one. The absolute size of these curvatures decreases with increasing concentrations indicating thus that both reciprocal relaxation times reach constant slopes at sufficiently high concentrations, where $1/\tau_1$ depends linearly on and $1/\tau_2$ is independent of $(\underline{c}_A+\underline{c}_B)$. The same conclusions are valid under the conditions where $c_{A0} \ll c_{B0}$ and the reciprocal relaxation times are plotted versus c_{B0}.

Model 3:

In the case of model 3 the derivatives are much more complicated than in the previous case. This is due to the fact that three coefficients a_{ij} now depend on concentrations (8). Therefore, comparatively simple expressions for the derivatives can only be derived if one reactant is present in excess. Based on $c_{A0} \ll c_{B0}$ the slope of the concentration dependence of both reciprocal relaxation times is given by (the upper sign corresponds to τ_1, the lower to τ_2):

$$\frac{d\,1/\tau_{1,2}}{d\,c_{B0}} = \frac{k_{12}+k_{23}}{2} \pm \frac{c_{B0}(k_{12}-k_{23})^2+k_{21}(k_{12}+k_{23})-k_{32}(k_{12}-k_{23})}{2\sqrt{c_{B0}^2(k_{12}-k_{23})^2+2c_{B0}[k_{21}(k_{12}+k_{23})-k_{32}(k_{12}-k_{23})]+(k_{21}-k_{32})^2}}\,. \tag{18}$$

In contrast to the case of model 2 this equation has two zeros, ${}^1c_{B0}$ and ${}^2c_{B0}$, at which the reciprocal relaxation times as function of c_{B0} exhibit relative extrema. They are determined by:

$${}^{1,2}c_{B0} = \frac{k_{32}(k_{12}-k_{23})-k_{12}(k_{12}+k_{23})[1\mp\sqrt{1-k_{32}/k_{21}(1-k_{23}/k_{12})}]}{(k_{12}-k_{23})^2}\,. \tag{19}$$

${}^1c_{B0}$ is attributed to the negative sign of the square root term in (19) and corresponds to the total concentration at which $1/\tau_2$ exhibits a relative extremum; accordingly ${}^2c_{B0}$ is obtained by the positive sign of the square root term and corresponds to the relative extremum of $1/\tau_1$.

The values of ${}^1c_{B0}$ and ${}^2c_{B0}$ must be real because they represent concentrations. Thus, the following condition must be fulfilled by the rate constants:

$$k_{32}/k_{21}(1-k_{23}/k_{12})<1\,. \tag{20}$$

If the rate constants are such that this inequality is fulfilled, relative extrema of both reciprocal relaxation times as a function of c_{B0} can occur in the case of model 3. Under this condition, however, the value of ${}^2c_{B0}$ is always negative, which is not meaningful for a concentration. For this reason, the dependence of $1/\tau_1$ versus c_{B0} cannot exhibit a relative extremum, which implies that this dependence must increase strictly monotonically. A relative extremum for the dependence of $1/\tau_2$ under this condition must occur in reality provided ${}^1c_{B0}$ is within the investigated c_{B0} range. If condition (20) is not fulfilled by the rate constants, no relative extrema can occur for model 3.

The second derivatives of the reciprocal relaxation times with respect to c_{B0} for $c_{B0} \gg c_{A0}$ are given by:

$$\frac{d^2\,1/\tau_{1,2}}{d\,c_{B0}^2} = \mp \frac{2k_{12}k_{21}^2k_{23}[1-k_{32}/k_{21}(1-k_{23}/k_{12})]}{\{c_{B0}^2(k_{12}-k_{23})^2+2c_{B0}[k_{21}(k_{12}+k_{23})-k_{32}(k_{12}-k_{23})]+(k_{21}-k_{32})^2\}^{3/2}}\,. \tag{21}$$

The negative sign corresponds to the curvature of $1/\tau_1$, the positive to that of $1/\tau_2$. As in the case of model 2 (cf. (17)) the sign of both curvatures is independent of c_{B0}. For sufficiently high concentrations the denominator in (21) increases with increasing c_{B0}. Therefore, the absolute size of the curvatures decreases wih increasing c_{B0} until constant slopes of the dependences of both reciprocal relaxation times on c_{B0} are reached.

If condition (20) is satisfied, the term in square brackets in the numerator of (21) is positive. Therefore, the curvature of $1/\tau_1$ is negative and that of $1/\tau_2$ is positive. Hence, if the dependence of $1/\tau_2$ on c_{B0} exhibits a relative extremum, it is a relative minimum. If the rate constants are such that condition (20) is not fulfilled, the numerator of (21) is negative and the signs of the curvature of both reciprocal relaxation times versus c_{B0} are interchanged. Under this condition, which excluded the occurrence of relative extrema, $1/\tau_1$ and $1/\tau_2$ are left- and right-curved functions respectively of c_{B0}, both for model 2 as well as for model 3.

Examples

In order to illustrate the considerations given above some representative examples concerning models 2 and 3 are shown by plotting the dependences of the reciprocal relaxation times versus either the total concentration of one or the sum of the free concentrations of both reactants. For this purpose, the sets of parameters compiled in Table 2 are chosen to calculate the dependences for both models. These parameters are related to a particular experimental situation (Bremer 1992) and are typical for the special, unfavourable case, where it is difficult to distinguish between models 2 and 3 on the basis of the resulting dependences, as originally anticipated. Figure 1 shows the dependence of the reciprocal relaxation times $1/\tau_1$ and $1/\tau_2$ as a function of c_{B0}, calculated according to (5) in combination with (7) or (8), respectively, employing the parameters given in Table 2 (for model 3 those of set a) were used. The concentration c_{B0} is chosen as ordinate for this figure because for most experimental studies the total concentration of one reactant is used in excess (here: $c_{B0} \gg c_{A0}$) so that $\underline{c}_B \approx c_{B0}$. If any simple arithmetic combination of the equilibrium concentrations of the reactants like $(\underline{c}_A + \underline{c}_B)$ were used instead of c_{B0}, the representations of the functions in Fig. 1 would not be changed significantly, which has been checked by additional simulations.

The c_{B0} range chosen for Fig. 1 covers two distinct ranges of well separated relaxation times (here: for $c_{B0} < 0.04$ M or $c_{B0} > 0.3$ M the relaxation times differ by at least a factor of 3), where an accurate determination of both times is possible (Strehlow and Knoche 1977), provided the corresponding relaxation amplitudes are not too different from each other. In addition a c_{B0} range is illustrated where the magnitudes of both relaxation times are similar (here: 0.04 M $\leq c_{B0} \leq$ 0.3 M).

If numerically identical parameters are used for model 2 as well as for model 3 (e.g. $k_{32} = 1 \times 10^4\ \mathrm{s}^{-1}$) the computer simulations performed in this study (data not shown) indicate that both models discussed here exhibit markedly different dependences of the reciprocal relaxation times on c_{B0}. Furthermore, the simulations indicate

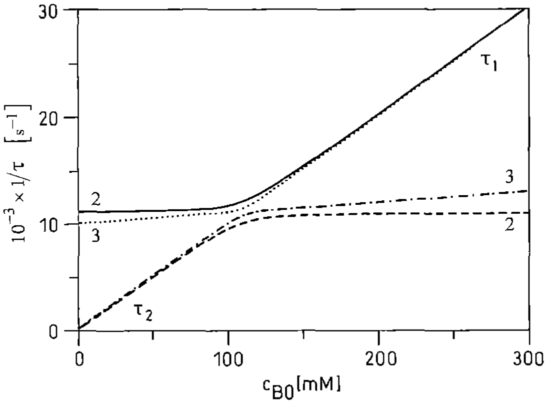

Fig. 1. Models 2 and 3: Plot of reciprocal relaxation times $1/\tau_1$ (— or ···) and $1/\tau_2$ (--- or -·-·) versus c_{B0} for the two-step binding models involving a rearrangement step (2) or a second binding step (3). The calculations were performed according to (5) in combination with (7) or (8), respectively, employing the parameters specified in Table 2 (for model 3 those of set a)). Other details are given in the text

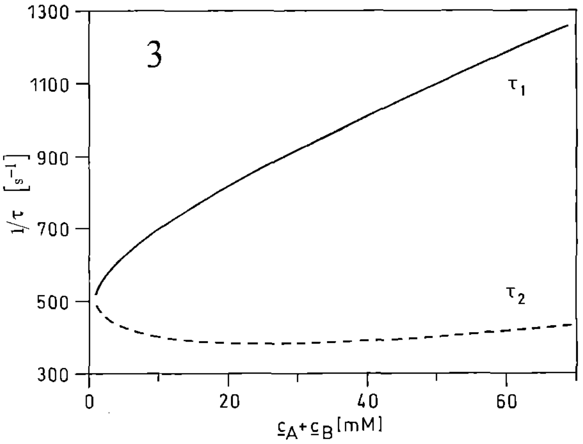

Fig. 2. Special case of model 3: Plot of the reciprocal relaxation times $1/\tau_1$ and $1/\tau_2$ versus the sum of the free equilibrium concentrations ($\underline{c}_A+\underline{c}_B$) according to (5) and (8). The parameters correspond to set b) in Table 2 with c_{B0} ranging from 1×10^{-4} to 7×10^{-2} M. Other details are mentioned in the text

Table 2. Parameters used for illustrating characteristic dependences of the reciprocal relaxation times as a function of reactant concentrations for reaction models 2 and 3, shown in Figs. 1 to 3

Parameter	Model 2	Model 3	
		Set a)	Set b)
c_{A0}	1×10^{-4} M	1×10^{-4} M	1×10^{-3} M
k_{12}	1×10^{5} $M^{-1} s^{-1}$	1×10^{5} $M^{-1} s^{-1}$	5×10^{3} $M^{-1} s^{-1}$
k_{21}	1×10^{2} s^{-1}	1×10^{2} s^{-1}	5×10^{2} s^{-1}
k_{23}	1×10^{4} s^{-1}	1×10^{4} $M^{-1} s^{-1}$	5×10^{3} $M^{-1} s^{-1}$
k_{32}	1×10^{3} s^{-1}	1×10^{4} s^{-1}	5×10^{2} s^{-1}

that in the case of model 2 the choice of other parameter combinations reveals graphs which are similar to those shown in Fig. 1. In contrast to this, if in the case of model 3 other rate constants or total concentrations than given in Table 2 set a) are chosen, the corresponding graphs of the reciprocal relaxation times versus c_{B0} may look different from those given in Fig. 1. The equations related to model 3 predict, over a wide c_{B0} range, linear graphs for both reciprocal relaxation times as long as the reciprocal stability constants $1/K_{12}$ and $1/K_{23}$ as well as c_{B0} are comparatively high compared to c_{A0}. Assuming $1/K_{12} \gg 1/K_{23}$, $c_{B0} \gg c_{A0}$ the shape of the curves is similar to that shown in Fig. 1.

As indicated by (18) a special situation can arise in the case of model 3: If both reciprocal stability constants $1/K_{12}$ and $1/K_{23}$ are of comparable magnitude and small, then a range of c_{B0} exists in the vicinity of c_{A0} in which $1/\tau_2$ decreases with increasing c_{B0}. The reciprocal relaxation times versus ($\underline{c}_A+\underline{c}_B$) shown in Fig. 2 represent typical examples for this situation under conditions where c_{B0} and c_{A0} are comparable. The parameters used for the calculations illustrated in Fig. 2 correspond to set b) in Table 2. Although k_{12} is numerically equal to k_{23} in this case, $1/\tau_2$ shows a minimum around $(\underline{c}_A+\underline{c}_B) = 25$ mM while (19) would predict this minimum at $(\underline{c}_A+\underline{c}_B) \to -\infty$ for a different experimental situation. This is due to the fact that the condition $c_{A0} \ll c_{B0}$, which was used to derive (19), is not satisfied under the circumstances chosen for calculating the dependences shown in Fig. 2.

In Fig. 3 the graphical representation of some of the approximations given in Table 1 (cases I and II for model 2 and case IV for model 2 and 3) are compared to those of the exact equations (5) in combination with either (7) or (8) for the set of parameters given in Table 2 (also used for Fig. 1). The other cases yield dependences of $1/\tilde{\tau}_i$ ($i = 1$ or 2) on c_{B0} which are similarly shaped to those shown in Fig. 3. From this figure one can get an idea about the c_{B0} range in which the equations given in Table 1 can be considered as reasonable approximations of the exact equations. This range is different for each investigated limiting case and depends on the involved rate constants as well as on c_{A0}. Large deviations between $1/\tilde{\tau}_i$ and $1/\tau_i$ are observed only at c_{B0} values for which the condition characterizing the corresponding limiting case is not fulfilled (for example, if the first reaction step does not equilibrate quickly compared to the second one as assumed in the case II, which is presented in Fig. 3b). Also in the cases shown in Fig. 3b, c and d, where the graph e.g. of $1/\tilde{\tau}_1$ approaches $1/\tau_2$ within a certain c_{B0} range, the conditions assumed for the deduction of the corresponding expressions given in Table 1 are not fulfilled.

In the case of strongly coupled equilibria of model 2 the corresponding $1/\tilde{\tau}_1$ and $1/\tilde{\tau}_2$ given in Table 1 (case I) approximate $1/\tau_1$ and $1/\tau_2$, respectively, reasonably well for the parameters listed in Table 2 only if c_{B0} significantly exceeds the value of 300 mM (Fig. 3a). The large deviations between $1/\tau_1$ and $1/\tilde{\tau}_1$ are mainly caused by the values of the rate constants of the rearrangement step. If the corresponding rate constants are smaller than assumed for the calculation of the dependences shown in Fig. 3a, the deviations become smaller. If model 2 is assumed under the special condition of weak coupling where the binding step equilibrates much faster than the subsequent rearrangement step (case II) and the rate con-

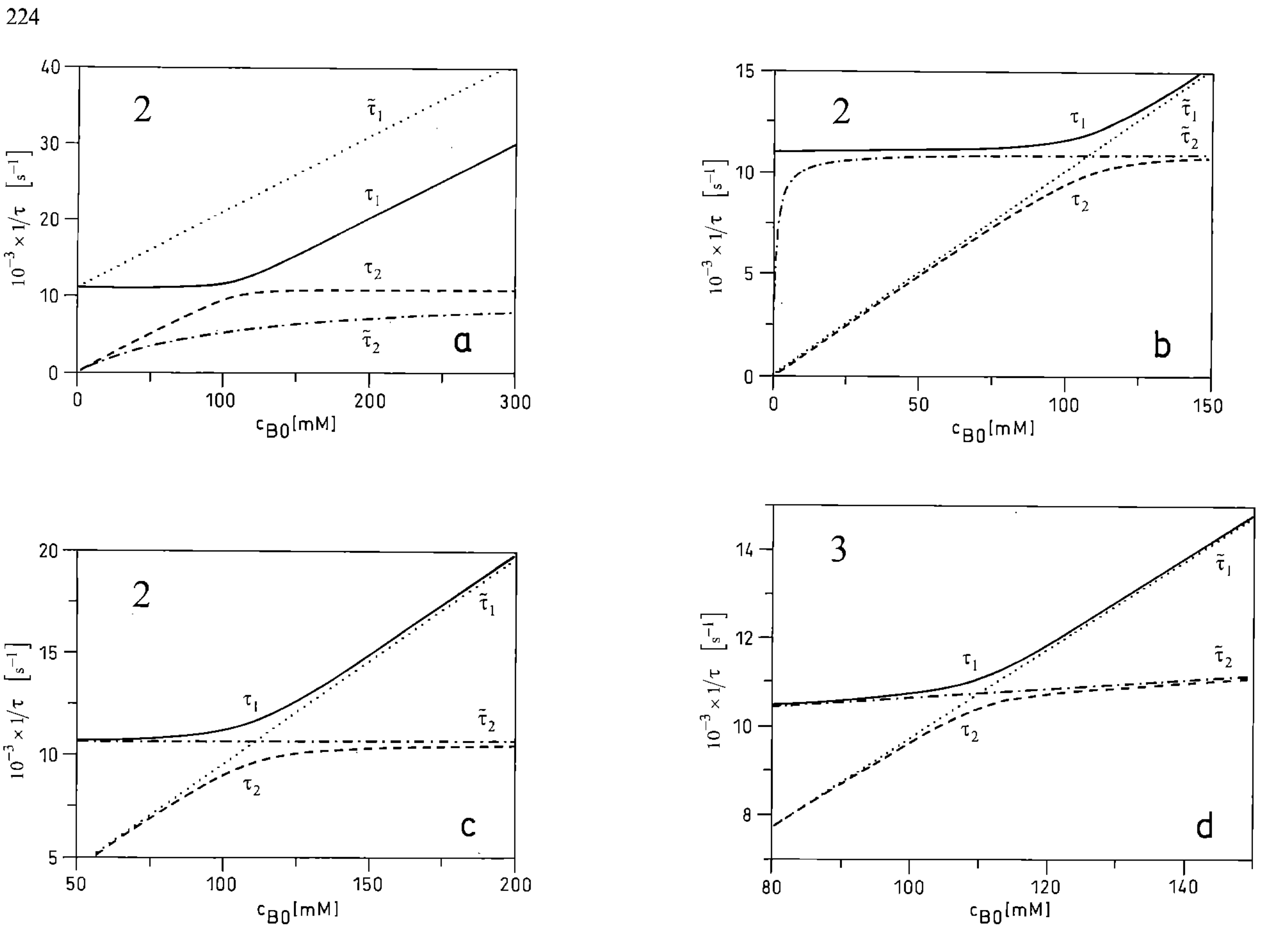

Fig. 3 a–d. Comparison of the exact dependences (5) of the reciprocal relaxation times as a function of c_{B0} (— for $1/\tau_1$; --- for $1/\tau_2$) with the corresponding approximate ones (Table 1; ··· for $1/\tilde{\tau}_1$; -·-· for $1/\tilde{\tau}_2$) for different experimental conditions. For the calculations the parameters given in Table 2 (set **a** in the case of model 3) were employed. A suitable scaling is chosen to emphasize clearly the differences between the exact dependences and the approximations. Model 2: **a** Both equilibria are assumed to be strongly coupled. **b** Weak coupling is assumed under conditions where the binding step equilibrates very rapidly compared to the rearrangement step. **c** No coupling between both equilibration steps. Model 3: **d** No coupling between both equilibration steps

stants as well as c_{A0} given in Table 2 are used for the corresponding calculations, then the $1/\tilde{\tau}_i$ for case II in Table 1 represent good approximations for the $1/\tau_i$ provided c_{B0} is larger than about 140 mM (Fig. 3b). If c_{B0} is below 70 mM, the $1/\tau_2$ is approximated by $1/\tilde{\tau}_1$ for case II in Table 1. If both equilibria are completely uncoupled and the parameters given in Table 2 are applied (set a for model 3), significant deviations are noticeable between the dependences of $1/\tau_i$ on c_{B0} and the corresponding $1/\tilde{\tau}_i$ given in Table 1 (case IV) for c_{B0} values below about 120 mM. At concentrations below about 100 mM the expressions for $1/\tilde{\tau}_1$ are good approximations of $1/\tau_2$ and vice versa. The c_{B0} range around 110 mM, where the exact dependences cannot be approximated by any of the expressions given for case IV, is larger in the case of model 2 (Fig. 3c) than in the case of model 3 (Fig. 3d). Within the same c_{B0} range the corresponding deviations are larger for model 2 than for model 3, too.

Figure 3 indicates also that $\tilde{\tau}_1 < \tilde{\tau}_2$ is true only under the condition of comparatively high concentrations at which $1/\tilde{\tau}_1$ and $1/\tilde{\tau}_2$ approach $1/\tau_1$ and $1/\tau_2$, respectively. In some of the examples shown in this figure the expressions compiled in Table 1 lead at rather low concentrations to $\tilde{\tau}_1 < \tilde{\tau}_2$ and approach reasonably well the dependences of exactly calculated reciprocal relaxation times, although the condition which was used to derive the corresponding approximative expressions is not met in this concentration range. This exemplifies the difficulty of relating the experimentally found relaxation times to calculated times $\tilde{\tau}_1$ and $\tilde{\tau}_2$.

Conclusions

Prior to any decision about the particular model for the reaction under investigation the assignment of the experimentally determined relaxation times to the theoretical ones must be performed. It can be achieved by means of the curvatures of the observed concentration dependences of the reciprocal relaxation times (17) and (21), respectively, by the existence of a relative extremum according to (18) or by the criteria given in the following for the four different limiting cases specified in Table 1.

Discrimination between models 2 and 3 under different experimental conditions

Only a single relaxation process is observable. A) $\tilde{\tau}_1$ is observable: The dependence of $1/\tilde{\tau}_1$ on suitable equilibrium concentrations is linear in most limiting cases discussed above (cf. dotted lines in Fig. 3). In order to distinguish between model 2 and 3 as well as to exclude the simple model 1, very special conditions must be met and experimental data of high precision are required. However, if under the given experimental conditions the second reaction step equilibrates slowly compared to the preceeding step (case II) or if both equilibria are completely uncoupled (case IV), no discrimination on the basis of $\tilde{\tau}_1$ is possible. If on the other hand $1/\tilde{\tau}_1$ is found to be concentration independent, model 3 can be excluded according to the expressions for $1/\tilde{\tau}_1$ given in Table 1. Thus, model 2 would apply under the condition that the second reaction step equilibrates much faster than the binding step (case III). In the case of strong coupling between both equilibria (case I), it is characteristic of model 3 that a deviation from a linear dependence of $1/\tilde{\tau}_1$ on $(\underline{c}_A + \underline{c}_B)$ must be observable, provided $\underline{c}_B$ is not large compared to $\underline{c}_A$ as well as to $\underline{c}_C$ and that a sufficiently large change of the ratio $\underline{c}_A/\underline{c}_C$ is achieved within a series of experiments. Model 2 predicts a strictly linear dependence of $1/\tilde{\tau}_1$ on $(\underline{c}_A + \underline{c}_B)$ for case I (cf. Fig. 3a).

B) τ_2 or $\tilde{\tau}_2$ is observable: This situation is frequently observed under conditions where the limited time resolution of a given experimental set-up does not allow one to determine the much shorter τ_1 with the corresponding relaxation process being indicated only by a fast amplitude change. Distinguishing models 2 and 3 by means of $1/\tau_2$, however, is much easier than on the basis of $1/\tau_1$. If in the case of model 3 $c_{B0} \gg c_{A0}$ and the condition (20) is met, the curvature of the dependence of $1/\tau_2$ is positive according to (21), while for model 2 this curvature always must be negative as indicated by (17). Considering the four limiting cases discussed above (cf. Table 1), the following possibilities exist to differentiate between model 2 and 3: I) If both equilibria are strongly coupled, model 2 predicts a monotonically rising dependence of $1/\tilde{\tau}_2$ versus $(\underline{c}_A + \underline{c}_B)$ ranging between the constant values corresponding to $k_{21} k_{32}/(k_{21} + k_{23} + k_{32})$ (for $(\underline{c}_A + \underline{c}_B) \ll 1/[K_{12}(1 + K_{23})]$, $1/K_{12} + k_{23}/k_{12}(1 + 1/K_{23})$) and $(k_{23} + k_{32})$ at very high concentrations (Fig. 3a). In contrast to this behaviour, for $c_{B0} \gg c_{A0}$ and strong coupling between both partial equilibria model 3 is characterized by a relative minimum of $1/\tilde{\tau}_2$ as a function of c_{B0} at $c_{B0} = -x + \sqrt{x^2 - 1/K_{23}(x - 1/K_{12})}$ with $x = (k_{21} + k_{32})/(k_{12} + k_{23})$ (existing only if $K_{12} < 1/x$) and by a linear increase with the slope $k_{12} k_{23}/(k_{12} + k_{23})$ at high c_{B0} values, as shown similarly in Fig. 2. II) If the first reaction step equilibrates rapidly compared to the subsequent one, it is more difficult to distinguish between models 2 and 3. Then, the major difference between the models on the basis of $\tilde{\tau}_2$ is detectable at high concentrations of species B: model 2 is then characterized by a constant value of $(k_{23} + k_{32})$ for $1/\tau_2$ (Fig. 3b), while the binding of a second molecule of B (model 3) results in a linear dependence of $1/\tilde{\tau}_2$ on c_{B0} with the slope k_{23} provided $c_{B0} \gg c_{A0}$ (similar to the situation shown in Fig. 1). III) A very high accuracy of the experimental data were necessary in the second case of weak coupling (if the second reaction step adjusts much faster compared to the first one), because then for both models exactly (model 2) or only partially (model 3) linear dependences are predicted over the whole or a large (c_{A0}, $c_{B0} \ll 1/K_{23}$ or $c_{B0} \gg c_{A0}$, $1/K_{12}$, $1/K_{23}$) range of $(\underline{c}_A + \underline{c}_B)$ values. This case, however, appears to be less relevant for the purpose of this model discrimination. IV) Another possibility to differentiate between the two models may be considered for the completely uncoupled case: According to model 2 no variation of $1/\tilde{\tau}_2$ is expected as a function of the concentrations involved, while for model 3 a linear of $1/\tilde{\tau}_2$ with increasing $(\underline{c}_C + \underline{c}_B)$ is predicted (Fig. 3 parts c and d).

Both relaxation processes are observable. If both relaxation processes are observable, in addition to the criteria discussed above for the individual relaxation times, the product of both reciprocal relaxation times can be used to discriminate between the models. If c_{B0} is at least comparable to $1/K_{23}$, but always large compared to c_{A0}, model 3 is characterized by a quadratic dependence on c_{B0} given by (15b), while model 2 leads to a linear dependence on $(\underline{c}_A + \underline{c}_B)$ under all possible conditions according to (11b).

Kinetic parameters obtainable for models 2 and 3 under different experimental conditions

Only a single relaxation process is observable. If only one relaxation process is resolvable and if one of the four limiting cases considered above applies throughout the whole concentration range examined experimentally, merely a subset of all four rate constants involved in model 2 and 3 can be determined (cf. Table 1). Only in the case of strong coupling and observation of $\tilde{\tau}_2$ can all individual rate constants be obtained. These situations will be discussed in detail in the next section.

In all other cases conditions are required where $1/\tau_1$ or $1/\tau_2$, respectively, depends non-linearly on the equilibrium concentrations according to the general expression (5). Since in this case τ_2 is longer than τ_1 for all possible concentrations, the experimentally found relaxation time can be assigned to either τ_1 or τ_2 (see above). Precise experimental data at different concentrations are required for the determination of all four rate constants of both reaction steps, because non-linear fitting procedures will have to be performed. The equilibrium concentrations involved in the Eq. (7) and (8) determining the coefficients a_{ij} are to be calculated from the total concentrations of the reactants, c_{A0} and c_{B0}, and the equilibrium constants given by the ratio of the rate constants of the corresponding reaction step. If the equilibrium constants are already known, the number of parameters to be determined by the fitting procedures can be reduced by a factor of two.

Both relaxation processes are observable. Concerning the four different cases discussed, the following conclusions can be drawn on the basis of the approximate expressions specified in Table 1: I) If both equilibria are strongly coupled, the evaluation of $1/\tilde{\tau}_1$ as function of $(\underline{c}_A+\underline{c}_B)$ yields only k_{12} as the slope and $(k_{21}+k_{23}+k_{32})$ as the intercept of the ordinate in the case of model 2. For model 3 such a simple evaluation is not possible, because $1/\tilde{\tau}_1$ as function $(\underline{c}_A+\underline{c}_B)$ is no longer represented by a straight line. According to the corresponding expression given in Table 1, the equilibrium concentration $\underline{c}_C$ is required in addition to $\underline{c}_A$ and $\underline{c}_B$. A fitting procedure then yields k_{12}, k_{23} and $(k_{21}+k_{32})$. Again in the case of strong coupling, $1/\tilde{\tau}_2$ depends for both models on all individual rate constants. If no further assumptions can be made, the dependences are rather complicated and require non-linear fitting procedures and the calculation of $\underline{c}_A$, $\underline{c}_B$ and $\underline{c}_C$ for the determination of the kinetic parameters. II) If the first reaction step adjusts much faster than the second one and if both relaxation processes are observed, all rate constants related to model 2 and 3 can be calculated. Under this condition of weak coupling the evaluation of the dependence of $1/\tilde{\tau}_1$ versus $(\underline{c}_A+\underline{c}_B)$ yields k_{12} and k_{21}; the corresponding dependence of $1/\tau_2$ reveals k_{23}, k_{32} and K_{12} involving different arithmetic combinations of equilibrium concentrations for model 3. III) If the first reaction step equilibrates much slower than the subsequent step, $\tilde{\tau}_1$ is attributed to the second partial reaction step. In this case all rate constants can be determined directly from the concentration dependences of $1/\tilde{\tau}_1$ and $1/\tilde{\tau}_2$ for model 3 only. If model 2 applies, k_{12}, $k_{21}/(1+K_{23})$ and $k_{23}(1+K_{23}) \equiv (k_{23}+k_{32})$ can in principle be calculated from the relaxation data obtained at different concentrations. In order to calculate the individual rate constants, K_{23} must be known in advance and thus has to be determined separately. IV) If both partial equilibria are completely uncoupled, all four rate constants can be calculated from $1/\tilde{\tau}_1$ and $1/\tilde{\tau}_2$ in the case of model 3. For model 2 it is only possible to determine k_{12} and k_{21} separately from the dependence of $1/\tilde{\tau}_1$; $1/\tilde{\tau}_2$ yields just the sum $(k_{23}+k_{32})$. Thus, again the equilibrium constant K_{23} has to be known prior to the calculation of the single rate constants.

If both relaxation times are resolvable within a suitable concentration range with comparably high precision, all rate constants can be calculated according to (10) from the coefficients of the corresponding polynomials which can be determined by a fit procedure from the sum and the product of the reciprocal relaxation times. Since the error of these functions is larger than the error of each of both single relaxation times, the corresponding coefficients and hence the calculated rate constants can be obtained only with rather low accuracy. If only one of both relaxation times is determined accurately, the expressions (10) are still useful for the purpose of qualitative model discrimination prior to a quantitative evaluation according either to (5) or the approximative expressions given in Table 1.

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