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ON THE ANALYSIS OF CHEMICAL RELAXATION AMPLITUDES

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The calculation of chemical relaxation amplitudes for a reaction mechanism involving an arbitrary number of thermodynamically independent steps is discussed. A formalism is developed which considerably reduces the computational labor required to derive expressions relating experimental amplitudes to thermodynamic functions and specific signal changes of elementary steps. The treatment includes the case of strong kinetic coupling between all reactions. The results are of particular interest in regard to the numerical fitting of amplitude data to an assumed mechanism.

1. Introduction

In an earlier paper [1] I pointed out that the results of Castellan's [2] formulation of chemical relaxation spectra could be used to greatly simplify the calculation of relaxation amplitudes. Subsequently this approach was further developed and applied to the interpretation of amplitude data for several multi-step reaction systems: the competitive binding between two inhibitors for the active site of trypsin [3]; the binding of proflavin to DNA [3,4]; the self-assembly of liver glutamate dehydrogenase [5]. The method differs from previous treatments [6–13] in that the amplitude expressions are given in a form which lends itself directly to numerical analyses with linear regression methods. The present report gives the mathematical basis of the new formalism as well as suggestions concerning its implementation.

2. Forced overall concentration shifts

The problem is to derive equations for a general mechanism which relate the overall concentration shifts brought about by a change of some external parameter (temperature, pressure or electric field strength) to the pertinent thermodynamic functions of individual steps and the reactant equilibrium concentrations.

We shall write the αth elementary step of a general reaction scheme as [2]

$$\sum_{i=1}^{N} \nu_{i\alpha} B_i = 0, \quad \alpha = 1, 2, ..., R, \tag{1}$$

where B_i is the ith chemical species, $\nu_{i\alpha}$ is the stoichiometric coefficient of species i in the αth reaction, N is the total number of chemical species and R is the total number of reaction steps. The stoichiometric coefficients are defined as negative for participants on the left side of an elementary reaction equation and positive for those on the right.

The concentration shifts of all species can be calculated from a consideration of R' thermodynamically independent reactions chosen from the total of R. A thermodynamically dependent reaction, σ, is one whose free energy change is a linear sum of the free energy changes of the thermodynamically independent set, or

$$G_\sigma = -\sum_{\beta=1}^{R'} s_{\beta\sigma} G_\beta, \quad \sigma = R'+1, R'+2, ..., R. \tag{2}$$

The $s_{\beta\sigma}$ are usually integers but may be fractions. Here however, we shall only treat those mechanisms where all steps are independent, or $R = R'$ [a].

By adopting Castellan's formalism, we may express the forced overall deviations of equilibrium concentrations as [2]

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[a] Jovin [14] has recently presented an elegant treatment of relaxation amplitudes for mechanisms involving thermodynamically dependent reactions, as well as new results on relations between concentration and advancement variables.

$$\Delta\bar{C}_i = (1/V) \sum_{\alpha=1}^{R} \nu_{i\alpha}\Delta\xi_\alpha, \tag{3}$$

where $\Delta\bar{C}_i$ is the total change of the molar equilibrium concentration of component i, V is volume and $\Delta\xi_\alpha$ is the net advancement of reaction α: $\Delta\xi = \xi - \bar{\xi}$. Bars denote equilibrium quantities. The N linear relations of eq. (3) are written in matrix form as

$$\mathbf{\Delta}\bar{\mathbf{C}} = (1/V)\,\mathbf{\nu}\mathbf{\Delta\xi}, \tag{4}$$

where $\mathbf{\Delta}\bar{\mathbf{C}}$ is an $N \times 1$ column vector of the $\Delta\bar{C}_i$'s, $1/V$ is a scalar, $\mathbf{\nu}$ is an $N \times R$ rectangular matrix whose typical element is $\nu_{i\alpha}$, and $\mathbf{\Delta\xi}$ is an $R \times 1$ column vector of the $\Delta\xi_\alpha$'s. Near equilibrium the free energy change of each reaction step can be expressed as the following sum of the independent $\Delta\xi$'s

$$G_\alpha = \sum_{\beta=1}^{R} G_{\alpha\beta}\Delta\xi_\beta. \tag{5}$$

When the activity coefficients are independent of the reactant concentrations (this can be achieved by fixing the ionic strength with a large excess of an inert electrolyte), the $G_{\alpha\beta}$ in eq. (5) simplify to $G_{\alpha\beta} = (RT/V)g_{\alpha\beta}$, where [2]

$$g_{\alpha\beta} = g_{\beta\alpha} = \sum_{i=1}^{N} \nu_{i\alpha}\nu_{i\beta}/\bar{C}_i. \tag{6}$$

The thermodynamic parameter of interest in chemical relaxation is $\Delta K_\alpha/K_\alpha$, which for small perturbations is proportional to a thermodynamic function of the αth elementary reaction.

"Temperature-jump"

$$\Delta K_\alpha/K_\alpha = (\Delta H_\alpha/RT)\Delta T/T, \qquad \Delta H_\alpha = \sum_{i=1}^{N} \nu_{i\alpha}\bar{H}_i, \tag{7}$$

$\bar{H}_i$ = partial molar enthalpy of species i.

"Pressure-jump"

$$\Delta K_\alpha/K_\alpha = -(\Delta V_\alpha/RT)\Delta P, \qquad \Delta V_\alpha = \sum_{i=1}^{N} \nu_{i\alpha}\bar{V}_i, \tag{8}$$

$\bar{V}_i$ = partial molar volume of species i, where the apparent equilibrium constant is defined as

$$K_\alpha = \prod \bar{C}_i^{\nu_{i\alpha}}, \tag{9}$$

Near equilibrium $\Delta K_\alpha/K_\alpha = G_\alpha/RT$, and we see from eq. (5) that the $\Delta K_\alpha/K_\alpha$'s are given by

$$\Delta\mathbf{K}/\mathbf{K} = (1/V)\mathbf{g}\mathbf{\Delta\xi}. \tag{10}$$

where $\Delta\mathbf{K}/\mathbf{K}$ is an $R \times 1$ column vector of the $\Delta K/K$'s and $\mathbf{g}$ is an $R \times R$ symmetrical matrix whose typical element is calculated from eq. (6).

It is of interest that the diagonal elements of matrix $\mathbf{g}$ can be conveniently written as the reciprocal Γ factors of the individual reaction steps [b]

$$g_{\alpha\alpha} = \sum_{i=1}^{N} (\nu_{i\alpha})^2/\bar{C}_i = 1/\Gamma_\alpha. \tag{11}$$

The typical off-diagonal element $g_{\alpha\beta}$ is a measure of the coupling between steps α and β, and contains the inverse concentrations of all components participating in both reactions. In the limit of high concentrations of these species the coupling between steps α and β becomes negligible and element $g_{\alpha\beta}$ can be set equal to zero.

From eq. (4) and eq. (10) we obtain N linear relations between the $\Delta\bar{C}_i$ and the set of independent thermodynamic functions:

$$\mathbf{\Delta}\bar{\mathbf{C}} = \mathbf{\nu}\mathbf{g}^{-1}\mathbf{\Delta K}/\mathbf{K}. \tag{12}$$

Matrix $\mathbf{g}^{-1}$ is the inverse of matrix $\mathbf{g}$. It will be noted that $\mathbf{g}^{-1}$ must exist since $\mathbf{g}$ has been defined in terms of a thermodynamically independent set of elementary reactions (i.e., the determinant of $\mathbf{g}$ is non-singular).

Since matrices $\mathbf{\nu}$ and $\mathbf{g}$ are constructed by inspection of the reaction stoichiometries, the $\Delta\bar{C}_i$ of a given model can be expressed in terms of equilibrium concentrations and individual thermodynamic functions without solving a large number of mass action and mass conservation relationships. The computational labor is thus reduced to the calculation of the inverse matrix $\mathbf{g}^{-1}$.

We recall that the typical element of $\mathbf{g}^{-1}$ is computed from the following equation

$$g^{-1}_{\alpha\beta} = g^{-1}_{\beta\alpha} = (-1)^{\alpha+\beta}|D_{\alpha\beta}|/|D|, \tag{13}$$

$|D|$ = determinant of $\mathbf{g}$.

The term $|D_{\alpha\beta}|$ is the determinant of the submatrix obtained by striking out the αth column and βth row of matrix $\mathbf{g}$. The determinant $|D|$ is immediately obtained from the $|D_{\alpha\beta}|$ by an expansion in terms of a single row (or column) of $\mathbf{g}$

$$|D| = \sum_{\beta=1}^{R} (-1)^{\alpha+\beta} g_{\alpha\beta}|D_{\alpha\beta}|. \tag{14}$$

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[b] The Γ factor characterizes the concentration shifts of a simple one-step reaction according to $\Delta\bar{C}_i/\nu_i = (\Delta K/K)\Gamma$.

It will be noted that due to the symmetry condition $g_{\alpha\beta} = g_{\beta\alpha}$, only $R\,(R+1)/2$ of the R^2 elements of $\mathbf{g}^{-1}$ need be calculated.

By means of eqs. (13) and (14) one can express the elements of $\mathbf{g}^{-1}$ in terms of reactant equilibrium concentrations and stoichiometric coefficients. Although these algebraic operations will admittedly be tedious for large matrices, the approach suggested here offers a considerably more straightforward and systematic means of calculating overall concentration shifts than the usual procedures [c]. It will also be noted that even for quite complex mechanisms the *numerical* inversion of $\mathbf{g}$ is easily realized with a digital computer.

3. Overall relaxation amplitudes

In a relaxation experiment one does not observe concentration shifts of individual chemical components, but rather changes of some measurable property of the system, P (P = light transmission, fluorescence intensity, conductivity, etc.), which is in general related to all $\Delta\bar{C}_i$. The overall relaxation amplitude, or the total signal change brought about by an external perturbation, is thus

$$\Delta P^0_{\text{tot}} = \sum_{i=1}^{N} \phi_i \Delta\bar{C}_i, \qquad \phi_i = \partial P/\partial\bar{C}_i. \tag{15}$$

where the "specific signals" ϕ_i are usually constants. If the measured property is light transmission, the "specific signal" ϕ_i is proportional to the molar extinction coefficient of the ith chemical component [d]. For small perturbations the $\Delta\bar{C}_i$ of eq. (15) are given in the relations of eq. (12). The quantity ΔP^0_{tot} may therefore be expressed in matrix form as

$$\Delta P^0_{\text{tot}} = \boldsymbol{\phi}^{\mathrm{T}} \boldsymbol{\upsilon}\, \mathbf{g}^{-1} \Delta K/K, \tag{16}$$

where $\boldsymbol{\phi}$ is an $N \times 1$ column vector of the ϕ_i's and T denotes transpose. Multiplication of $\boldsymbol{\upsilon}$ by $\boldsymbol{\phi}^{\mathrm{T}}$ in the above equation gives

$$\Delta P^0_{\text{tot}} = \Delta\boldsymbol{\phi}^{\mathrm{T}} \mathbf{g}^{-1} \Delta K/K, \tag{17}$$

where $\Delta\boldsymbol{\phi}$ is an $R \times 1$ column vector whose typical element is the "specific signal change" of the αth elementary step.

$$\Delta\phi_\alpha = \sum_{i=1}^{N} \nu_{i\alpha} \phi_i. \tag{18}$$

The quantity $\Delta\phi_\alpha$ is formally analogous to ΔH_α and ΔV_α [cf. eqs. (7) and (8)], and after normalization with respect to instrument constants may be considered a physical parameter characterizing the αth elementary reaction. Finally, expansion and rearrangement of eq. (17) yields the following general expression for the overall amplitude

$$\begin{array}{rl} \Delta P^0_{\text{tot}} = b_{11} g^{-1}_{11} & + b_{12} g^{-1}_{12} + \ldots + b_{1R} g^{-1}_{1R} \\ & + b_{22} g^{-1}_{22} + \ldots + b_{2R} g^{-1}_{2R} \\ & \qquad \ddots \qquad\qquad \vdots \\ & \qquad\qquad\qquad + b_{RR} g^{-1}_{RR} \end{array} \tag{19}$$

where

$$b_{\alpha\alpha} = \Delta\phi_\alpha \, \Delta K_\alpha / K_\alpha,$$

$$b_{\alpha\beta} = \Delta\phi_\alpha \, \Delta K_\beta / K_\beta + \Delta\phi_\beta \, \Delta K_\alpha / K_\alpha. \tag{20}$$

The simple form of eq. (19) is particularly useful for calculating the overall amplitude of an assumed model. In the present formalism the overall amplitude is in general the sum of $R\,(R+1)/2$ terms written in a triangular array. Each term contains a concentration-independent and concentration-dependent factor. The former [eq. (20)] are functions of elementary physical and thermodynamic parameters and are calculated by examination of the reaction stoichiometries [e]. The concentration-dependent factors are simply the elements of the inverse matrix $\mathbf{g}^{-1}$.

Eq. (19) is of considerable interest in regard to fitting an assumed mechanism to overall amplitude data.

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[c] General formulas may be derived for the elements of the inverse matrix $\mathbf{g}^{-1}$ in terms of the $g_{\alpha\beta}$. In this sense overall concentration shifts may be calculated directly from the stoichiometry of the model.

[d] Eq. (15) assumes that the specific signals are insensitive to the thermodynamic forcing function and that volume changes are negligible. When important, these physical effects can often be suppressed electronically with an automatic balancing system, or can be taken into account by introducing correction terms in the amplitude equations. It is emphasized that the ϕ_i of eq. (15) are functions of both physical parameters and instrument factors. In absorption measurements $\phi_i = l\epsilon_i$, where l denotes optical path length and ϵ_i is the molar extinction coefficient of species i.

[e] In some applications (such as turbidity measurements) the ϕ_i of eq. (15) may be concentration dependent. The formalism of eq. (19) would then be modified by grouping together the concentration dependent terms of the $\Delta\phi_\alpha$ and the corresponding $g^{-1}_{\alpha\beta}$.

In practice one would determine $\Delta P^0_{\mathrm{tot}}$ over a wide range of reactant concentrations. If the elements of $\mathbf{g}^{-1}$ can be estimated with sufficient precision, eq. (19) becomes a multiple linear regression equation in which $\Delta P^0_{\mathrm{tot}}$ is the dependent variable, the elements of $\mathbf{g}^{-1}$ are the independent variables and the b's are the regression coefficients to be determined. If all $\Delta\phi$ and $\Delta K/K$ values are significant, and if all concentration variables are linearly independent, a conventional least-squares treatment of the overall amplitude data will yield estimates of the $R(R+1)/2$ regression coefficients together with their standard errors. The b's obtained in the statistical treatment allow one to calculate a set of theoretical amplitudes corresponding to the "best fit" for the assumed mechanism.

If a satisfactory fitting is obtained, one can attempt to estimate the elementary $\Delta\phi$ and $\Delta K/K$ parameters from the experimental regression coefficients. This phase of the analysis requires information from an independent experiment, and the details of such calculations are discussed later in this paper. It is emphasized, however, that with the above procedure a mechanism can be fit to amplitude data without any knowledge of elementary specific signal changes or thermodynamic functions.

4. Individual relaxation amplitudes

For small perturbations the rate of equilibration of a complex reaction system is in general described by a sum of exponential decays. Each exponential is defined by a relaxation time τ and an individual relaxation amplitude. The number of exponentials is equal to the number of thermodynamically independent steps of the mechanism, or

$$\Delta P(t) = \sum_{n=1}^{R'} \Delta P^0_n \exp(-t/\tau_n). \tag{21}$$

If the relaxation times are widely separated on the time axis, individual amplitudes can be calculated by a straightforward extension of the formalism developed in the preceding section.

We first consider those mechanisms where all elementary steps are independent ($R = R'$) and equilibrate at very different rates (an order of magnitude difference between successive relaxation times will usually be sufficient). Until now in our discussion, the assignment of numbers to the elementary steps has been entirely arbitrary. Here, however, we begin by numbering the elementary steps according to their relative rates of equilibration, $\alpha = 1, 2, ..., R$, where reactions 1 and R are respectively the fastest and slowest elementary steps. The $R \times R$ matrix $\mathbf{g}_R$ ($\mathbf{g}_R \equiv \mathbf{g}$) is then constructed by inspection of the mechanism. Next the $R-1$ sub-matrices of $\mathbf{g}_R$ are written as

$$\mathbf{g}_r = \begin{bmatrix} g_{11} & g_{12} \cdots & g_{1r} \\ g_{21} & g_{22} \cdots & g_{2r} \\ \vdots & \vdots & \vdots \\ g_{r1} & g_{r2} \cdots & g_{rr} \end{bmatrix} \tag{22}$$

and the corresponding inverse matrices $\mathbf{g}_r^{-1}$ are calculated. If, as assumed here, the condition $\tau_{r-1} \ll \tau_r \ll \tau_{r+1}$ is satisfied, the rth individual amplitude is to a good approximation given by

$$\Delta P^0_r = (\Delta P^0_{\mathrm{tot}})_r - (\Delta P^0_{\mathrm{tot}})_{r-1}, \tag{23}$$

where $(\Delta P^0_{\mathrm{tot}})_r$ and $(\Delta P^0_{\mathrm{tot}})_{r-1}$ are the total signal changes calculated by neglecting steps $r+1$ through R and steps r through R, respectively. Expressions for these variables are readily obtained by setting $R = r$ and $R = r-1$ in eq. (19). It then follows that the rth individual amplitude is given by the following sum of $r(r+1)/2$ terms,

$$\begin{aligned} \Delta P^0_r = {} & b_{11}[(g^{-1}_{11})_r - (g^{-1}_{11})_{r-1}] \\ & + b_{12}[(g^{-1}_{12})_r - (g^{-1}_{12})_{r-1}] + \cdots + b_{1r}(g^{-1}_{1r})_r \\ & + b_{22}[(g^{-1}_{22})_r - (g^{-1}_{22})_{r-1}] + \cdots + b_{2r}(g^{-1}_{2r})_r \\ & \qquad\qquad\qquad \ddots \qquad\qquad \vdots \\ & \qquad\qquad\qquad\qquad\qquad + b_{rr}(g^{-1}_{rr})_r \end{aligned} \tag{24}$$

where the coefficients are given in eq. (20) and the concentration-dependent factors are differences between corresponding elements of matrices $\mathbf{g}_r^{-1}$ and $\mathbf{g}_{r-1}^{-1}$. Both overall and individual relaxation amplitudes are therefore calculated and analyzed statistically by means of very similar formalisms.

5. Normal relaxation amplitudes

When the relaxation times of a system are widely separated, one can calculate individual amplitudes from thermodynamic relationships only. When two or more relaxation times are of the same order of magnitude, however, a more general treatment is required.

Near equilibrium, exact relaxation amplitude equations can be derived by means of a "normal mode" analysis [6,8–13]. In this approach a completely decoupled set of R "normal" advancements may be defined:

$$\Delta\xi_r^*(t) = \Delta\xi_r^* \exp(-t/\tau_r), \quad r = 1, 2, \ldots, R, \tag{26}$$

where the asterisk denotes normal parameters and the condition $R = R'$ is assumed. The transformation matrix Q relates the normal and actual advancements,

$$\boldsymbol{\Delta\xi} = Q\boldsymbol{\Delta\xi}^*, \tag{27}$$

where $\boldsymbol{\Delta\xi}$ and $\boldsymbol{\Delta\xi}^*$ are $R \times 1$ column vectors of the independent $\Delta\xi$'s and $\Delta\xi^*$'s respectively. Matrix Q is constructed from the eigenvectors of the matrix of rate coefficients rg, which is calculated by examination of the mechanism (2).

The rth normal advancement is related to a normal Γ factor and normal enthalpy change (or normal volume change) by

$$\Delta\xi_r^* = \Gamma_r^* \Delta \ln K_r^*; \quad \Delta \ln K_r^* = \Delta H_r^* (\Delta T/RT^2). \tag{28}$$

Recently Schimmel [11] pointed out that one can obtain the normal enthalpy and volume changes by simply transposing Q:

$$\boldsymbol{\Delta H}^* = Q^{\mathrm{T}} \boldsymbol{\Delta H}, \quad \boldsymbol{\Delta V}^* = Q^{\mathrm{T}} \boldsymbol{\Delta V}, \tag{29}$$

where $\boldsymbol{\Delta H}^*$, $\boldsymbol{\Delta V}$, etc. are $R \times 1$ column vectors of the ΔH^*'s, ΔV's, etc. Schimmel has also shown that the normal Γ factors are the reciprocal elements of a diagonal matrix D, where $D = Q^{\mathrm{T}} \mathrm{g} Q$. Although the normal parameters ΔH^* (or ΔV^*) and Γ^* define the amplitudes obtained with a stationary technique (e.g., sound absorption), they are not sufficient for an analysis of amplitudes observed with transient methods (temperature-jump, pressure-jump or electric-field jump). It is therefore of interest to extend Schimmel's useful formalism to the more general case.

To this end we may define a set of normal specific signal changes, $\Delta\phi_\alpha^*$, by the equation

$$\Delta P_{\mathrm{tot}}^0 = (1/V) \boldsymbol{\Delta\phi}^{*\mathrm{T}} \boldsymbol{\Delta\xi}^*, \tag{30}$$

where $\boldsymbol{\Delta\phi}^*$ is an $R \times 1$ column vector of the $\Delta\phi^*$'s. It follows from eq. (10) and eq. (17) that the observed overall amplitude may be expressed as

$$\Delta P_{\mathrm{tot}}^0 = (1/V) \boldsymbol{\Delta\phi}^{\mathrm{T}} \boldsymbol{\Delta\xi}. \tag{31}$$

If the relation of eq. (27) is substituted into the above equation, we obtain

$$\Delta P_{\mathrm{tot}}^0 = (1/V) \boldsymbol{\Delta\phi}^{\mathrm{T}} Q \boldsymbol{\Delta\xi}^*. \tag{32}$$

It then follows that

$$\boldsymbol{\Delta\phi}^* = Q^{\mathrm{T}} \boldsymbol{\Delta\phi}. \tag{33}$$

Thus the transformation of eq. (29) also gives the normal specific signal changes.

We now write the exact expression for the rth experimental amplitude as

$$\Delta P_r^0 = \Gamma_r^* \Delta\phi_r^* \Delta H_r^* (\Delta T/RT^2). \tag{34}$$

By means of eqs. (7), (29) and (33), the normal parameters $\Delta\phi_r^*$ and ΔH_r^* of eq. (34) can be given in terms of elementary $\Delta\phi$'s and $\Delta K/K$'s. After expansion and rearrangement we obtain

$$\begin{aligned} \Delta P_r^0 = \Gamma_r^* (b_{11} Q_{1r}^2 + b_{12} Q_{1r} Q_{2r} &+ \ldots + b_{1R} Q_{1r} Q_{Rr} \\ + b_{22} Q_{2r}^2 &+ \ldots + b_{2R} Q_{2r} Q_{Rr} \\ &\ddots \quad \vdots \\ &+ b_{RR} Q_{Rr}^2) \end{aligned} \tag{35}$$

where the b's are given in eq. (20) and the $Q_{\alpha r}$'s are the elements of the rth column of the transformation matrix Q.

We have thus cast the exact equation for the rth individual amplitude in the convenient form characterizing overall and widely separated amplitudes, but now the concentration-dependent terms are the products $\Gamma_r^* Q_{\alpha r} Q_{\beta r}$, where $\beta = 1, 2, \ldots, R$ and $\alpha \leqslant \beta$. The possibility of analyzing experimental amplitudes with a multiple linear regression is again evident. However, since the independent variables of the regression equations now contain specific rate constants of the mechanism, a kinetic study is a necessary preliminary to an amplitude analysis [15]. It can be added that the experimental resolution of strongly coupled relaxation phenomena is often difficult.

If the relaxation times of a system are widely separated, limiting ratios between specific rate constants can be used to reduce the exact amplitude equations to expressions which are free of kinetic parameters. However, in these conditions eq. (24) provides a less laborious and more straightforward means of computing amplitudes. Of course if specific rate constants are at hand, one should substitute the results of the approximate amplitude treatment into the exact expressions and verify that the assumption of negligible kinetic coupling is valid.

6. Experimental determination of elementary equilibrium constants, specific signal changes and thermodynamic functions

It is clear that elementary equilibrium constants must be known in order to calculate the elements of matrix $\mathbf{g}$. Moreover, in linear regression applications the precision of the equilibrium constants must be such that the uncertainty in the independent variables are small compared to the experimental errors in the amplitudes.

The determination of elementary equilibrium constants by static methods is usually not possible where monomolecular processes intervene. If, however, the monomolecular steps of a mechanism are kinetically coupled to one or more second-order reactions, a complete kinetic analysis by relaxation methods is in principle feasible; individual equilibrium constants can then be estimated from the specific rate constants.

In many cases elementary equilibrium constants can be determined directly from relaxation amplitudes by non-linear regression. In this approach the elements of the inverse matrices $\mathbf{g}_r^{-1}$ are expressed in terms of analytical concentrations and equilibrium constants and the amplitude data are fit to eqs. (19) and (24) with an iterative procedure which finds the best set of equilibrium constants for a particular model. When feasible, this method yields both b's and equilibrium constants from amplitude data alone. Iterative amplitude analyses of one- and two-step reaction systems have recently appeared [16,17].

The number of specific signal changes and thermodynamic functions which can be evaluated from relaxation amplitudes depends on the stoichiometry of the system and on the amount of independent information available. Let us first assume that the stoichiometry is such that all concentration terms of eq. (19) are linearly independent. From the relations of eq. (20) we derive the following independent set of $R(R-1)/2$ quadratic equations.

$$(\Delta\phi_\alpha\, \Delta K_\beta/K_\beta)^2 - b_{\alpha\beta}(\Delta\phi_\alpha\, \Delta K_\beta/K_\beta) + b_{\alpha\alpha}b_{\beta\beta} = 0, \tag{36}$$

where $\beta = 1, 2, ..., R$ and $\alpha < \beta$. The $\Delta\phi_\alpha\, \Delta K_\beta/K_\beta$ can therefore be calculated from the experimental b's by means of the following formula [f],

$$\Delta\phi_\alpha\, \Delta K_\beta/K_\beta = [b_{\alpha\beta} + (b_{\alpha\beta}^2 - 4b_{\alpha\alpha}b_{\beta\beta})^{1/2}]/2. \tag{37}$$

In addition, there are R relations of the form $b_{\alpha\alpha} = \Delta\phi_\alpha\, \Delta K_\alpha/K_\alpha$. Thus in the general case where all $\Delta\phi$ and $\Delta K/K$ values are significant, the experimental b's will yield estimates of $R(R+1)/2$ independent products $\Delta\phi_\alpha\, \Delta K_\beta/K_\beta$, where $\beta = 1, 2, ..., R$, and $\alpha \leqslant \beta$:

$$\begin{array}{llll} \Delta\phi_1 \Delta K_1/K_1, & \Delta\phi_1 \Delta K_2/K_2, & ..., & \Delta\phi_1 \Delta K_R/K_R, \\ & \Delta\phi_2 \Delta K_2/K_2, & ..., & \Delta\phi_2 \Delta K_R/K_R, \\ & & \ddots & \vdots \\ & & & \Delta\phi_R \Delta K_R/K_R. \end{array} \tag{38}$$

To pass from the above set of products to the elementary $\Delta\phi$'s and $\Delta K/K$'s, an additional experimental parameter is required. The independent information could be a single $\Delta\phi$ or $\Delta K/K$ value, but often one cannot determine these quantities by usual experimental methods. On the other hand, thermodynamic functions and specific signal changes of overall reactions are accessible to classical techniques, and may provide the necessary data for a complete solution.

If monomolecular reactions figure in the mechanism, linear relations will exist between elements of $\mathbf{g}_R^{-1}$. Eq. (19) should then be reduced to the minimum number of linearly independent terms. Monomolecular processes therefore place limitations on the number of elementary parameters obtained from overall amplitudes, just as in "classical" thermodynamic measurements. On the other hand, monomolecular reactions may, but do not necessarily reduce the number of independent terms characterizing the *individual* amplitude expressions of eq. (24).

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[f] The remaining root of eq. (36) is $\Delta\phi_\beta\, \Delta K_\alpha/K_\alpha$.

Regarding optical detection, it is of considerable interest that $\Delta\phi$'s determined at different wavelengths may yield absolute optical spectra of reaction intermediates. In this sense chemical relaxation data contain structural as well as kinetic and thermodynamic information. Even if the products of eq. (38) cannot be solved for individual parameters, ratios such as $\Delta\phi_1/\Delta\phi_R$, $\Delta\phi_2/\Delta\phi_R$, ... 1, can be computed, which constitute a series of relative difference spectra.

Finally, with the present formalism one can quickly examine the consequences of allowing specific signal changes and thermodynamic functions to approach limiting values. Consider the example of a mechanism in which only one component gives rise to a signal and this component participates only in the slowest reaction step. It is then clear from eq. (20) that all $b_{\alpha\beta} = 0$ ($\alpha \leqslant \beta$) except where $\beta = R$. If the relaxation times are widely separated we conclude from eq. (24) that only ΔP_R^0 will be observed and we see immediately that the amplitude equation is

$$\Delta P_R^0 = b_{1R}(g_{1R}^{-1})_R + b_{2R}(g_{2R}^{-1})_R + \ldots + b_{RR}(g_{RR}^{-1})_R . \qquad (39)$$

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