

# Kinetic Parameter Estimation by Numerical Algorithms and Multiple Linear Regression: Theoretical

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**Abstract** □ A new method is presented for the determination of kinetic parameters based on a functional relationship among experimental data derived from the postulated model. The data, even though containing errors, are manifestations of this relationship, which should be satisfied by parameters fitted to the system. The procedure involves the use of numerical integration and/or differentiation of the data, followed by multiple linear regression. It does not require initial estimates or repetitive iteration for linear systems and can be applied to nonlinear models. The accuracy of the estimated parameter depends on the goodness of the particular numerical approximation method used.

**Keyphrases** □ Kinetic parameters—estimated by numerical algorithms and multiple linear regression □ Models, mathematical—kinetic parameters estimated by numerical algorithms and multiple linear regression

The estimation of parameter values is essential in the analysis of many chemical kinetic, biochemical kinetic, and pharmacokinetic processes. The procedure involves the initial development of a model with the corresponding mathematical equations. The latter are then fitted, in a least-squares sense, to the experimental data by adjusting the parameter values.

Computer programs were developed (1, 2) using nonlinear regression procedures with modified Gauss-Newton methods (3, 4). The subject of nonlinear optimization methods was reviewed previously (5) and will not be elaborated here. Because of inherent difficulties associated with the nonlinear fitting procedure, such as the requirement of good initial estimates and lengthy computations, other alternative methods were developed. A method was described (6) in which the differential (rate) equations are approximated by Picard's polynomials, while the experimental data are approximated by least-squares orthogonal polynomials. The parameters are then computed by equating the matching coefficients of the corresponding series. In this method, the computation time is greatly reduced by sacrificing some accuracy of the parameter estimates.

A new procedure involves numerical differentiation by fitting smoothed least-squares polynomials to the data (7). The parameters are calculated algebraically from rate equations and then further improved by a nonlinear optimization method. Because of the inherent difficulty in obtaining reliable derivatives, occasional divergence of the results occurs. The method is applicable to models where all dependent variables are experimentally measured. Other special methods include one for a biexponential equation where data points are equally spaced (8).

The present paper describes a new general alternative method using linear regression analysis. The method is sufficiently flexible and applicable to many linear and nonlinear systems.

## THEORETICAL

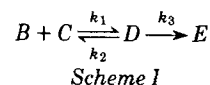
The principle of the method lies in the fact that experimental obser-

vations, if error free, are not random outcomes. They are functionally related and are themselves manifestations of this relationship. This relationship is dictated by the empirical equations proposed to be consistent with the data.

In reality, experimental data inevitably contain measurement errors, so manifestations of the relationships may be distorted or completely obscured. However, if errors are within some arbitrary tolerable limits, the relationships may still be discernible in the observed data and should be describable by parameters fitted to the system.

The mathematical procedures include the use of numerical integration and/or differentiation of the experimental data as well as algebraic substitution of entities not observed. The use of numerical approximation methods is an attempt to reconstruct complete time profiles based on available information since most kinetic data are often obtained and presented as discrete data points. These manipulations are linear, and the resultant solution can be expressed as a linear function of a new set of independent variables generated by data transformation. Because of this linearity, they become amenable to the method of multiple linear regression for parameter optimization. The following examples illustrate the principle and procedures of the proposed method.

**Case I**—In the reaction system shown in Scheme I:



reactants  $B$  and  $C$  form a reversible intermediate  $D$ , which is then converted into product  $E$ . The rate equations are:

$$\dot{B} = \dot{C} = -k_1BC + k_2D \quad (\text{Eq. 1})$$

$$\dot{D} = k_1BC - (k_2 + k_3)D \quad (\text{Eq. 2})$$

$$\dot{E} = k_3D \quad (\text{Eq. 3})$$

with the initial values and mass balance constraints of:

$$B(0) = B_0 \quad (\text{Eq. 4a})$$

$$C(0) = C_0 \quad (\text{Eq. 4b})$$

$$D(0) = E(0) = 0 \quad (\text{Eq. 4c})$$

$$B(0) = B + D + E \quad (\text{Eq. 5a})$$

$$C(0) = C + D + E \quad (\text{Eq. 5b})$$

where  $B$ ,  $C$ ,  $D$ , and  $E$  are functions of time  $t$  and the dot accent denotes a first-time derivative.

Mathematically, Eqs. 1-3 represent a linear transformation from  $[B, C, D]^T$  into  $[\dot{B}, \dot{C}, \dot{D}, \dot{E}]^T$  as follows:

$$\begin{bmatrix} \dot{B} \\ \dot{C} \\ \dot{D} \\ \dot{E} \end{bmatrix} = \begin{bmatrix} -k_1 & k_2 \\ -k_1 & k_2 \\ k_1 & -(k_2 + k_3) \\ 0 & k_3 \end{bmatrix} \begin{bmatrix} B \\ C \\ D \end{bmatrix} \quad (\text{Eq. 6})$$

If  $B$  and  $C$  are assumed to be experimental observations, the unknown variable  $D$  in Eq. 1 can be eliminated by using Eqs. 3 and 5a (or 5b):

$$\dot{B} = (k_2 + k_3)[B(0) - B] - k_1BC - k_1k_3 \int_0^t BC dt \quad (\text{Eq. 7})$$

Upon integration, Eq. 7 gives:

$$B = B(0) + (k_2 + k_3) \left[ B(0)t - \int_0^t B dt \right] - k_1 \int_0^t BC dt - k_1k_3 \int_0^t \int_0^t BC dt dt \quad (\text{Eq. 8})$$

In regression form, Eq. 8 may be written as:

$$B = A_0 + A_1X_1 + A_2X_2 + A_3X_3 \quad (\text{Eq. 9})$$

where  $A_j$ 's are regression coefficients,  $X_j$ 's are independent variables<sup>1</sup>, and:

$$A_0 = B(0) \quad (\text{Eq. 10a})$$

$$A_1 = k_2 + k_3 \quad (\text{Eq. 10b})$$

$$A_2 = -k_1 \quad (\text{Eq. 10c})$$

$$A_3 = -k_1k_3 \quad (\text{Eq. 10d})$$

$$X_1 = B(0)t - \int_0^t B dt \quad (\text{Eq. 11a})$$

$$X_2 = \int_0^t BC dt \quad (\text{Eq. 11b})$$

$$X_3 = \int_0^t \int_0^t BC dt dt \quad (\text{Eq. 11c})$$

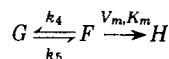
Equation 9 shows that the dependent variable  $B$  can be expressed as a linear function of the three independent variables, which can be estimated from observations  $B$  and  $C$  by any suitable numerical integration method. When appropriate weighting factors are assigned to the data points, the intercept and the three coefficients can be obtained by multiple linear regression. The intercept represents the fitted initial value, and the three coefficients can be used to compute the three model parameters.

Equation 8 indicates that the experimental observations are not functions of time alone but are also the cumulative behavior of all previous events. Therefore, it is important that the experimental data points be strategically located to minimize errors in numerical approximation. The variable  $C$  can be further eliminated by substitution if it is not an experimental observation.

The present method does not consider each model parameter as a separate entity. The parameters are grouped together to form hybrid regression coefficients. When partial derivatives of the weighted sum of residuals with respect to coefficients are taken, the resultant normal equations are linear in the coefficients, yielding a unique set of coefficient values. By appropriate data transformation, the number of coefficients can be adjusted to equal the number of model parameters so that the resultant parameter values are also unique to the system.

An important task in the present method is to obtain reliable numerical estimates of the independent variables. The trapezoidal method tends to produce systematic errors due to the nature of linear data interpolation. Polynomial interpolation has been successfully implemented in these laboratories using spline functions (9). Specifically, local data are fitted with serial cubic polynomial functions for sections of data that are theoretically differentiable. Other methods, including the Lagrange method, are available (10, 11).

**Case II**—For some systems, the procedure may involve direct scanning of parameters that are not linearly related. Consider the reaction shown in Scheme II:



Scheme II

in which the reactant  $F$  forms the product  $H$  via Michaelis-Menten-type kinetics. Concurrently, the formation of intermediate  $G$  is reversible and first order. The reaction may be written as:

$$\dot{F} = -k_4F + k_5G - \frac{V_mF}{K_m + F} \quad (\text{Eq. 12})$$

$$\dot{G} = k_4F - k_5G \quad (\text{Eq. 13})$$

$$\dot{H} = \frac{V_mF}{K_m + F} \quad (\text{Eq. 14})$$

where  $V_m$  is the maximum rate;  $K_m$ , the nonlinear parameter of the system, is the Michaelis constant having the same dimensions as  $F$ .

With the following initial values and the mass balance constraints:

$$F(0) = F_0 \quad (\text{Eq. 15a})$$

$$G(0) = H(0) = 0 \quad (\text{Eq. 15b})$$

$$F(0) = F + G + H \quad (\text{Eq. 16})$$

Eq. 12 can be transformed into:

$$F = \frac{k_5F(0)}{(k_4 + k_5)} - \left[ \frac{1}{(k_4 + k_5)} \right] \dot{F} - \left[ \frac{V_m}{(k_4 + k_5)} \right] \left[ \frac{F}{(K_m + F)} \right] - \frac{V_mk_5}{(k_4 + k_5)} \int_0^t \frac{F dt}{(K_m + F)} \quad (\text{Eq. 17})$$

or, in regression form:

$$F = A_0 + A_1X_1 + A_2X_2 + A_3X_3 \quad (\text{Eq. 18})$$

where:

$$A_0 = \frac{k_5F(0)}{(k_4 + k_5)} \quad (\text{Eq. 19a})$$

$$A_1 = \frac{-1}{(k_4 + k_5)} \quad (\text{Eq. 19b})$$

$$A_2 = \frac{-V_m}{(k_4 + k_5)} \quad (\text{Eq. 19c})$$

$$A_3 = \frac{-V_mk_5}{(k_4 + k_5)} \quad (\text{Eq. 19d})$$

$$X_1 = \dot{F} \quad (\text{Eq. 20a})$$

$$X_2 = \frac{F}{(K_m + F)} \quad (\text{Eq. 20b})$$

$$X_3 = \int_0^t \frac{F}{(K_m + F)} dt \quad (\text{Eq. 20c})$$

Values for  $X_1$  can be obtained by numerical differentiation of the local data using spline interpolation or the polynomial smoothing technique (7). Numerical differentiation is a difficult procedure and requires great care to obtain reliable results. Values for  $X_2$  can be computed at each data point by assigning an arbitrary constant value to  $K_m$ . Values for  $X_3$  can be then estimated by numerical integration of  $X_2$ . Thus, for each assumed  $K_m$  value, the coefficients in Eq. 18 can be obtained by regression analysis after appropriate weights are assigned to the data.

The corresponding  $F_0$  and the unknown constants  $k_4$ ,  $k_5$ , and  $V_m$  can be computed from Eqs. 19a–19d. By using this set of parameters, including  $K_m$ , the theoretical values of  $F$  can be generated by any suitable means such as the Runge-Kutta method. The goodness of the fit can be measured by computing the weighted sum of the squares of residuals,  $SS$ , and scanning the  $SS$  values as a function of  $K_m$ . Such a plot represents a projection of the  $SS$  surface on the  $K_m$  coordinate and has a unique minimum; it yields a corresponding set of best fit parameter values.

**Case III**—For a large body of kinetic data described by a nonspecific multiexponential equation of the form:

$$Y = \sum_{i=1}^n a_i \exp(-\lambda_i t) \quad (\text{Eq. 21})$$

the intercept parameter  $a_i$  and the exponent parameter  $\lambda_i$  can be similarly estimated by using multiple integrals of  $t$  and  $Y$  (also see Appendix). This result is due to the special properties of the equation in that its derivatives and integrals are again multiexponential equations. For example, if  $n = 3$ , there are six undetermined constants and the regression equation takes the form:

$$Y = \sum_{j=1}^6 A_j X_j \quad (\text{Eq. 22})$$

where:

$$A_1 = a_1 + a_2 + a_3 \quad (\text{Eq. 23a})$$

$$A_2 = -q_1A_5 + q_2A_6 \quad (\text{Eq. 23b})$$

$$A_3 = -q_1A_6 \quad (\text{Eq. 23c})$$

$$A_4 = -(\lambda_1 + \lambda_2 + \lambda_3) \quad (\text{Eq. 24a})$$

$$A_5 = -(\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1) \quad (\text{Eq. 24b})$$

$$A_6 = -(\lambda_1\lambda_2\lambda_3) \quad (\text{Eq. 24c})$$

$$X_1 = 1.0 \quad (\text{Eq. 25a})$$

$$X_2 = t \quad (\text{Eq. 25b})$$

<sup>1</sup> Although  $X_j$ 's are obtained by data transformation, they are considered independent variables so long as they are not linearly dependent.

$$X_3 = \frac{1}{2!} t^2 \quad (\text{Eq. 25c})$$

$$X_4 = \int_0^t Y dt \quad (\text{Eq. 25d})$$

$$X_5 = \int_0^t \int_0^t Y dt dt \quad (\text{Eq. 25e})$$

$$X_6 = \int_0^t \int_0^t \int_0^t Y dt dt dt \quad (\text{Eq. 25f})$$

$$q_1 = (a_1/\lambda_1) + (a_2/\lambda_2) + (a_3/\lambda_3) \quad (\text{Eq. 26a})$$

$$q_2 = (a_1/\lambda_1^2) + (a_2/\lambda_2^2) + (a_3/\lambda_3^2) \quad (\text{Eq. 26b})$$

The three exponent parameters can be calculated from coefficients  $A_4$ ,  $A_5$ , and  $A_6$ , as shown in Eqs. 24a–24c, by finding the roots of the cubic equation:

$$x^3 + A_4x^2 - A_5x + A_6 = 0 \quad (\text{Eq. 27})$$

General methods of finding roots of  $n$ th-degree polynomial equations can be found in many text books (10, 12). Computer subroutines are also available (13). A cubic equation can also be solved by using the trigonometric method (14). The three intercept parameters can be obtained by solving Eqs. 23a, 26a, and 26b, which represent a system of three simultaneous equations in three unknowns where  $q_1$  and  $q_2$  can be computed from Eqs. 23b and 23c.

## DISCUSSION

The goodness of the estimated parameters depends on the accuracy of the calculated  $X_j$  values. For a given set of  $X_j$  values, the proposed method yields a unique set of parameter values. For this reason, it is important to choose a reliable empirical function that can best numerically approximate the true, but unknown, function. Of the several numerical approximation methods, such as least-squares polynomials (7), the Akima method (11), spline functions (15, 16), or modified splines (17), spline functions have been tested and found to be a convenient procedure (16).

Splines are ideal in the estimation of areas because the errors they generate are usually small and less biased and the effects of fluctuation in input data are usually dampened by numerical integration. However, since some  $X_j$  values are obtained by data transformation, they are not error free. The errors are related to those of the input data. Currently, the statistical aspect of the present method is under investigation.

While integration is a smoothing operation, numerical differentiation is a rather hard and undesirable one. It tends to magnify even small noises in the data. Thus, when it is used to obtain  $X_1$  values of Eq. 17, large errors should be anticipated. The least-squares polynomials (7) are usually more satisfactory but do not completely eliminate the problem. Because of the unreliability, application of numerical differentiation should be monitored carefully or avoided whenever possible. For example, the  $\bar{F}$  in Eq. 17 may be eliminated by further integration to yield a new regression equation after rearrangement. In any event, the estimated parameters may be refined further by using a nonlinear regression procedure.

In nonlinear regression, parameters are often searched by iteration in confined parameter spaces to prevent meaningless results. On the other hand, no constraints are imposed on parameters in the present method. Thus, unexpected solutions, such as negative rate constants, may occur, but this result does not imply that the method is inadequate. Rather, it may suggest that the model is inadequate for the data or that large and/or systematic errors have been introduced in some calculated  $X_j$  values. In cases such as multiexponential equations, such inconsistencies may lead to the formation of  $\lambda_i$  parameters in conjugated complex form.

Implicit in the application of the proposed method is that each data point should be single valued so that the corresponding  $X_j$  values are also single valued after numerical integration or differentiation. For replicate observations, the data should be fitted with smoothing least-squares polynomials (7) or least-squares splines as described by Wold (15). The fitted curve can be used to calculate the required  $X_j$  values.

In essence, the present method is predicated on the recognition that, for a given kinetic system, there is one unique functional relationship among all experimental data. The data are conceptually tied together to form one or more continuous strings and then utilized in one regression step to obtain the best fit parameters. Therefore, the algorithm is greatly simplified, resulting in high efficiency in data processing. The estimated parameters are also comparable to those obtained by other more com-

plicated methods. A recent report (18) indicated that parameters obtained by the present method agreed well with those obtained by Pfeffer (19), using the spectinomycin pharmacokinetic data of Wagner *et al.* (20).

Another advantage is that solutions to many linear systems, which may be difficult to obtain, are not required. For nonlinear systems where explicit solutions are not available, the use of the Runge-Kutta procedure does not represent a new complicating factor in the present method since it is a well-established technique.

## APPENDIX

This section describes the derivation of the regression equation discussed in Case III. The procedure is general and can be applied to any multiexponential equations.

In connection with Eq. 21, which simplifies to:

$$Y = \sum a_i \exp(-\lambda_i t) \quad (\text{Eq. A1})$$

the following notations are defined:

$$q_1 = \sum (a_i/\lambda_i) \quad (\text{Eq. A2})$$

$$q_2 = \sum (a_i/\lambda_i^2) \quad (\text{Eq. A3})$$

$$q_3 = \sum (a_i/\lambda_i^3) \quad (\text{Eq. A4})$$

⋮

$$q_n = \sum (a_i/\lambda_i^n) \quad (\text{Eq. A5})$$

$$s_1 = \sum \lambda_i \quad (\text{Eq. A6})$$

$$s_2 = \sum_{i \neq k} \lambda_i \lambda_k = \text{sum of product of } \lambda_i \text{ taken two at a time} \quad (\text{Eq. A7})$$

$$s_3 = \text{sum of product of } \lambda_i \text{ taken three at a time} \quad (\text{Eq. A8})$$

⋮

$$s_n = \prod \lambda_i \quad (\text{Eq. A9})$$

$$Q_1 = \sum (a_i/\lambda_i) \exp(-\lambda_i t) \quad (\text{Eq. A10})$$

$$Q_2 = \sum (a_i/\lambda_i^2) \exp(-\lambda_i t) \quad (\text{Eq. A11})$$

$$Q_3 = \sum (a_i/\lambda_i^3) \exp(-\lambda_i t) \quad (\text{Eq. A12})$$

⋮

$$Q_n = \sum (a_i/\lambda_i^n) \exp(-\lambda_i t) \quad (\text{Eq. A13})$$

$$R_1 = \int_0^t Y dt \quad (\text{Eq. A14})$$

$$R_2 = \int_0^t R_1 dt \quad (\text{Eq. A15})$$

$$R_3 = \int_0^t R_2 dt \quad (\text{Eq. A16})$$

⋮

$$R_n = \int_0^t R_{n-1} dt \quad (\text{Eq. A17})$$

As can be seen, both  $q_i$ 's and  $s_i$ 's are time-independent constants while  $R_i$ 's and  $Q_i$ 's are functions of time  $t$ . In addition,  $R_i$ 's are obtained by successive integration and are related to  $Q_i$ 's as follows:

$$R_1 = q_1 - Q_1 \quad (\text{Eq. A18})$$

$$R_2 = q_1 t - q_2 + Q_2 \quad (\text{Eq. A19})$$

$$R_3 = \frac{1}{2} q_1 t^2 - q_2 t + q_3 - Q_3 \quad (\text{Eq. A20})$$

$$R_n = \frac{1}{(n-1)!} q_1 t^{n-1} - \frac{1}{(n-2)!} q_2 t^{n-2} + \dots + (-1)^{n-1} q_n + (-1)^n Q_n \quad (\text{Eq. A21})$$

In the case of  $n = 2$ , Eqs. A6–A9 yield:

$$S_1 = \lambda_1 + \lambda_2 \quad (\text{Eq. A22})$$

$$S_2 = \lambda_1 \lambda_2 \quad (\text{Eq. A23})$$

Multiplying both sides of Eq. A18 by  $(1/\lambda_1 + 1/\lambda_2)$  yields:

$$R_1 \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) = q_1 \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) - Q_1 \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) \quad (\text{Eq. A24})$$

$$R_1(\lambda_1 + \lambda_2)/(\lambda_1\lambda_2) = q_2 + (a_1 + a_2)/(\lambda_1\lambda_2) - (R_2 - q_1t + q_2) - Y/(\lambda_1\lambda_2) \quad (\text{Eq. A25})$$

or:

$$Y = (a_1 + a_2) + (\lambda_1\lambda_2)q_1t - (\lambda_1 + \lambda_2)R_1 - (\lambda_1\lambda_2)R_2 \quad (\text{Eq. A26})$$

In regression form, Eq. 26 may be written as:

$$Y = \sum_{j=1}^4 A_j X_j \quad (\text{Eq. A27})$$

where  $A_1 = a_1 + a_2$ ,  $A_2 = -q_1A_4$ ,  $A_3 = -(\lambda_1 + \lambda_2)$ ,  $A_4 = -(\lambda_1\lambda_2)$ ,  $X_1 = 1.0$ ,  $X_2 = t$ ,  $X_3 = \int_0^t Y dt$ , and  $X_4 = \int_0^t X_3 dt$ .

This procedure may be applied to other cases. For example, if  $n = 3$ , then:

$$s_1 = \lambda_1 + \lambda_2 + \lambda_3 \quad (\text{Eq. A28})$$

$$s_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1 \quad (\text{Eq. A29})$$

and:

$$s_3 = \lambda_1\lambda_2\lambda_3 \quad (\text{Eq. A30})$$

Multiplying Eq. A19 by  $(1/\lambda_1 + 1/\lambda_2 + 1/\lambda_3)$  yields:

$$R_2 \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} + \frac{1}{\lambda_3} \right) = (q_1t - q_2) \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} + \frac{1}{\lambda_3} \right) + Q_2 \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} + \frac{1}{\lambda_3} \right) \quad (\text{Eq. A31})$$

which, when expanded, gives:

$$\begin{aligned} \frac{S_2 R_2}{S_3} &= \frac{S_2}{S_3} (q_1t - q_2) - (R_3 - \frac{1}{2} q_1t^2 + q_2t - q_3) \\ &+ \left( \frac{1}{\lambda_2} + \frac{1}{\lambda_3} \right) (a_1/\lambda_1^2) \exp(-\lambda_1t) + \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_3} \right) (a_2/\lambda_2^2) \exp(-\lambda_2t) \\ &+ \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right) (a_3/\lambda_3^2) \exp(-\lambda_3t) \quad (\text{Eq. A32}) \end{aligned}$$

Equation A32 is simplified, yielding:

$$s_2 R_2 = s_2(q_1t - q_2) - s_3(R_3 - \frac{1}{2} q_1t^2 + q_2t - q_3) + s_1(q_1 - R_1) - Y \quad (\text{Eq. A33})$$

or:

$$Y = (a_1 + a_2 + a_3) + (s_2q_1 - s_3q_2)t + \frac{1}{2} s_3q_1t^2 - s_1R_1 - s_2R_2 - s_3R_3 \quad (\text{Eq. A34})$$

Equation A34 can be written in the following regression form:

$$Y = \sum_{j=1}^6 A_j X_j \quad (\text{Eq. A35})$$

where  $A_1 = a_1 + a_2 + a_3$ ,  $A_2 = -A_5q_1 + A_6q_2$ ,  $A_3 = -A_6q_1$ ,  $A_4 = -(\lambda_1 + \lambda_2 + \lambda_3)$ ,  $A_5 = -(\lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1)$ ,  $A_6 = -(\lambda_1\lambda_2\lambda_3)$ ,  $X_1 = 1.0$ ,  $X_2 = t$ ,  $X_3 = \frac{1}{2} t^2$ ,  $X_4 = \int_0^t Y dt$ ,  $X_5 = \int_0^t X_4 dt$ , and  $X_6 = \int_0^t X_5 dt$ .

Thus, Eq. A35 is identical to Eq. 22 in the text. By induction, the regression equation can be proved to take the following general form:

$$Y = \sum_{j=1}^{2n} A_j X_j \quad (\text{Eq. A36})$$

where:

$$\begin{aligned} A_1 &= \sum a_i \\ A_2 &= -q_1A_{n+2} + \dots + (-1)^{n-1}q_{n-1}A_{2n} \\ A_3 &= -q_1A_{n+3} + \dots + (-1)^{n-2}q_{n-2}A_{2n} \\ &\vdots \\ A_n &= -q_1A_{2n} \end{aligned}$$

$$\begin{aligned} A_{n+1} &= -s_1 \\ A_{n+2} &= -s_2 \\ &\vdots \\ A_{2n} &= -s_n \\ X_1 &= 1.0 \\ X_2 &= t \\ X_3 &= t^2/2! \\ &\vdots \\ X_n &= t^{n-1}/(n-1)! \\ X_{n+1} &= \int_0^t Y dt \\ X_{n+2} &= \int_0^t X_{n+1} dt \\ &\vdots \\ X_{2n} &= \int_0^t X_{2n-1} dt \end{aligned}$$

Equation A36 has some distinct properties. First, there are two series in  $X_j$ ; consecutive integrals of  $t$  and those of  $Y$ . Analogously,  $A_j$  can also be separated into two series: (a) functions of both the intercept parameters  $a_i$  and exponent parameters  $\lambda_i$ , and (b) strictly functions of  $\lambda_i$  only. Finally, the first term  $A_1X_1$  in Eq. A36 can be viewed as the intercept of the fitted curve. In a special situation where the multiexponential function is constrained to pass through zero, the regression equation contains only  $2n - 1$  terms rather than  $2n$  terms. This is due to the disappearance of the  $A_1X_1$  term since  $A_1$  is forced to be identically zero, and there are only  $2n - 1$  unknown parameters in the original multiexponential equation.

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## ACKNOWLEDGMENTS AND ADDRESSES

Received March 25, 1976, from Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

Accepted for publication February 16, 1977.

The author thanks Dr. K. C. Kwan for many valuable suggestions.