volume of the drug. Under the conditions where shape, size, weight, degree of compression, and surface area remain essentially unchanged, the above equation reduces to:

$$Q = kt^{1/2} \qquad \text{(Eq. 2)}$$

where k is a constant taking account of all the factors held constant in Eq. 1.

The percent of SETD released from the tablets after dissolution in the acid pepsin medium from all three matrices evaluated in this investigation was plotted as a function of square root of time. The linear relationship according to Eq. 2 was observed to be valid for a few hours where the release was less than 2%. After that the dissolution data did not follow this pattern, apparently due to considerable change in the effective surface area of the tablets. Similarly, the percent of SETD released from the tablets after dissolution in alkaline pancreatin medium from all three matrices was plotted as a function of square root of time. Likewise, in this instance, a deviation from linearity was observed after a few hours due to change in effective surface area of the tablets as erosion and some dispersion of the tablets occurred. Tablets compressed from spray-congealed products containing white wax alone as the matrix were observed to be more soluble than the other wax matrices employed and, hence, showed more deviation. After 6 hr. of exposure in the alkaline pancreatin medium, the SETD–white wax tablets were about half of the original size.

Dissolution of SETD from Spray-Congealed SETD–Wax Powders *versus* Dissolution of SETD from Tablets—It would be of interest to compare the dissolution of SETD from spray-congealed SETD–wax powders to the dissolution of SETD from tablets prepared from the same products. Figure 1 shows the percent of SETD-released against time from SETD-synthetic waxlike ester–white wax, in a 1 to 1 proportion, powder and tablets. It is apparent that the magnitude of release of SETD from the powdered forms is much greater than that in the tablet dosage form. The compressional force required in tableting resulted in some fusion, less porosity, and more compactness of the particles which seems to be responsible for the small amount of SETD release from the tablets.

Further work on tablets employing spray-congealed products of drug in wax matrices with modifier, which are free flowing, suitable for direct compression, and which release the active ingredient more completely, is presently under investigation. The results of this study will be reported separately in a later paper.

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Fitting a Double-Exponential Curve to Observed Salicylate Concentrations in Blood

F. W. MUELLER and S. V. LIEBERMAN

Abstract □ For interpretation of the results of blood concentration data to be meaningful, a very careful evaluation of the basic aspects of data collection, data description, and analysis is essential. The importance of these considerations is illustrated by the magnitude of observed differences in rate constants obtained under a variety of possible data-handling methods. The method of curve fitting presented, which minimizes squared logarithmic deviations, offers a different approach by utilizing relative error rather than absolute error. If truly equal weights are desired for data points, it is felt that this is the more appropriate definition of best fit. In any case, no mathematical technique for fitting a model to the data can compensate for an inadequate description of drug activity.

Keyphrases □ Blood concentration data—evaluation, basic aspects □ Rate constants—double-exponential curve fitting □ Salicylate concentration levels—rate constants, curve fitting, example

A desire to determine rate constants for drug absorption and elimination has resulted in the development of numerous analytical and mathematical techniques for pharmacokinetic analysis. In recent years, the applications of pharmacokinetic analysis have progressed rapidly from graphical solutions of concentration *versus* time plots to computer programs applied to increasingly sophisticated mathematical models. The latter yield apparent first-order rate constants for various processes of distribution and elimination [*e.g.*, Levy *et al.* (1) and Wagner (2)]. In many publications, the estimated values obtained for the parameters of the models have been presented without any indication that other values are possible. Where a number of apparent first-order rate constants are derived by a series of arithmetic manipulations from these estimated parameters, any inaccuracy in these estimates will be magnified in the subsequent computations. For a given set of observations, there are several important statistical considerations which merit careful attention before beginning the process of fitting a specific model to observed data.

STATISTICAL CONSIDERATIONS

An equation, describing the biological system, is used as a model, and an iterative curve-fitting procedure is necessary to obtain the estimated rate constants. This requires: (*a*) a selection of a descriptive statistic for the data, (*b*) a definition of best fit, and (*c*) a choice of the method of deriving the best fit. All three affect the estimated equation and, as a result, the conclusions drawn from an evaluation of the results. The average is the statistic most frequently used to describe concentration levels for a group of individuals. The best fit is commonly defined as the curve which minimizes the sum of the squared deviations between observed and computed concentrations. Under certain conditions, however, other alternatives are equally rational.

The selection of a statistic should obviously be based upon its descriptive quality. Physiological characteristics of individuals are such that, many times, salicylate concentration levels at a given time are not normally distributed. Consequently, the median of a group may be a better choice than the mean. If drug concentrations at observed time periods follow the normal or any other symmetric distribution, results with mean or median will be very similar. As for best fit, either the sum of the squared differences between observed and computed concentrations or the sum of the squared differences between the logarithms of these concentrations can be minimized. Comparisons of these latter differences are equivalent to relative percent deviations. Usually, accuracy of analytical methods is in terms of percentage error rather than absolute error, and a definition of best fit using differences in logarithms may be more appropriate. As basic as all these considerations seem, very little attention has been given to how they affect results.

In order to estimate the kinetic rate constants for orally administered drugs in tablet form, a biexponential curve-fitting procedure was developed for Teorell's equation 25 (3) for two consecutive monomolecular reactions giving the relationship of drug concentration in blood with time. Both averages and medians were used in fitting, and results based on each of the previously mentioned definitions of best fit were compared. The purposes of this paper are: to present this fitting procedure; to discuss briefly other available methods; and to illustrate the effect of data point selection, the definition of best fit, and the descriptive statistic chosen on the estimated rate constants.

METHODS AND DISCUSSION

The Model—Teorell's equation 25, describing the kinetics of drug appearance in the blood and disappearance from the blood when administered other than intravenously, was used:

$$C = \frac{a_0 Ka}{Vd(Ka - Kd)}\left(e^{-Kdt} - e^{-Kat}\right) = \frac{\gamma Ka}{\delta}\left(e^{-Kdt} - e^{-Kat}\right) \qquad \text{(Eq. 1)}$$

where: C = blood drug concentration in mcg./ml.; a_0 = dose in mg./kg.; Vd = the specific apparent volume of distribution (l./kg.); Ka = apparent drug appearance rate constant in hr.$^{-1}$; Kd = apparent drug disappearance rate constant in hr.$^{-1}$; t = time after ingestion in hours; γ = proportionality constant (a_0/Vd); and $\delta = Ka - Kd$.

In the manner of Lowenthal and Vitsky (4), rearranging Eq. 1 gives Eq. 2,

$$C = \frac{\gamma Ka}{\delta} e^{-Kat}\left(e^{\delta t} - 1\right) \qquad \text{(Eq. 2)}$$

and taking natural logs of Eq. 2 gives the linear Eq. 3:

$$\ln \frac{C}{e^{\delta t} - 1} = \ln \frac{\gamma Ka}{\delta} - Kat \qquad \text{(Eq. 3)}$$

The Delta Search—A least-squares best fit for Eq. 3 is derived by a search of delta (δ) values from an initially estimated δ in steps of 0.1. For each delta, a residual sum of squares about the fitted line is obtained. Once the direction of delta, from the initial estimate is determined, the 0.1 increment or decrement is repetitively applied for as long as a decrease in variability is observed. When an increase occurs, the search step is reduced to 0.005 and the value of the delta which gives the best fit is obtained. The absolute value of the slope of the best fit line is the estimated Ka. Subtracting δ from Ka yields Kd, and by substituting the Ka and Kd values the intercept ($\ln \gamma\, Ka/\delta$) can be solved for gamma (γ).

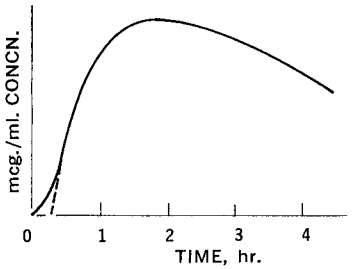

Figure 1—*Typical salicylate concentration curve for aspirin, 650 mg. (10 gr.).*

Time Axis Intercept—When the dose is administered as tablets, a form which does not provide immediate availability of the complete dose, early moments are not characterized by the first-order biexponential model. Instead, a time gap exists between the time of administration (time zero) and an extrapolated "kinetic time zero," the time at which the best fitting curve crosses the time axis. Termination of the fitting process at time zero assumes that the model is immediately applicable upon administration of the drug, and that the extrapolated curve should pass through the origin. As illustrated by Fig. 1, this is not true. The best fit to the observed data is based on a curve passing through the time axis at a point greater than zero.

The next phase of the search is directed toward finding the time axis intercept which gives the overall best fit. This is accomplished by subtracting a time increment, in this case 1 min., from each of the observed time periods, and once again searching for a delta to obtain the best fit to the data. The squared deviations for this fit and the previous fit are compared. If the current fit is superior, an additional time increment is subtracted and the delta search is repeated. This is continued until the sum of squared deviations for the current fit shows an increase. At this point, 0.5-min. time increments are applied, and the estimate of the time axis intercept is determined to the nearest half minute (Fig. 2). The search is now complete and an optimum Ka, Kd, γ, and time axis intercept (Δt) are available.[1]

Best Fit—The procedure just described was designed to obtain a best fit by minimizing the sum of squared logarithmic deviations between observed and computed concentrations. Three papers, Lowenthal and Vitsky (4), Wagner (5), and Wiegand and Sanders (6), have presented other mathematical techniques for fitting the double-exponential model. All of these published methods are designed to obtain a set of constants minimizing the sum of squares for deviations between observed and computed concentrations.

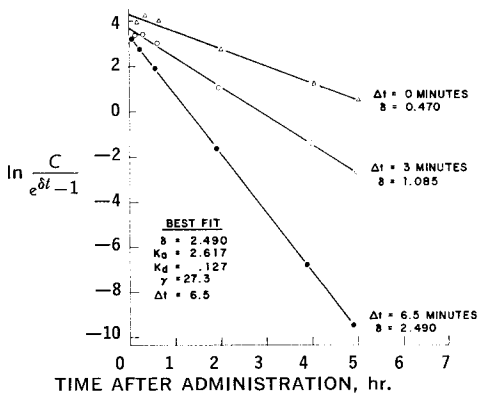

Figure 2—*Illustration of delta search for time axis intercept* (Δt). *Data points are from average salicylate concentration following 650-mg. (10-gr.) dose of aspirin. Best fit results are* $\delta = 2.490$, Ka = *2.617*, Kd = *0.127*, $\gamma = 27.3$, *and* $\Delta t = 6.5$.

[1] This computer program will be supplied upon request.

Table I—Computed Concentrations (mcg./ml.) from Best Fit Curve with and without 10-min. Data Point

Time, min.	Buffered Aspirin, 650 mg. (10 gr.)—Averages—With 10-min. Data Point	Observed Average Concn.	Excluding 10-min. Data Point
10	5.87	5.88	7.95
20	19.21	19.20	19.19
40	25.79	25.63	25.64
120	23.52	23.45	23.67
240	18.95	19.58	18.96
300	17.01	16.64	16.99
	Ka = 5.61		Ka = 4.91
	Kd = 0.108		Kd = 0.111
	γ = 28.3		γ = 28.6
	Δt = 7.5		Δt = 6.0

Selections of a definition of best fit, of observation times for data points, and of a descriptive statistics are, to a degree, a matter of choice and certainly subject to more than one opinion. The evaluation of which method provides the best fit is much more objective. For a given definition of best fit, the method can be considered correct only if it generates a set of constants meeting the least-squares criterion, *i.e.*, minimal residual sum of squares.

A valid comparison of these methods would require the application of each program to a common set of data. Although this has not been done, a general review of these procedures suggests that the results would differ. The Lowenthal and Vitsky method uses both definitions of best fit during the search for the constants. A two-dimensional grid search for *Ka* and *Kd* is performed. For each *Ka* and *Kd*, a gamma to complete the set is computed from the intercept of the best fit to the linearized Eq. 3 previously shown. The best set of constants is selected on the basis of minimal sum of squares of differences in observed and computed concentrations. Obtaining gamma by one definition of best fit and the best set by the other definition gives a different result than one from a three-dimensional search.

Wagner submits too little detail to evaluate the iterative routine for estimating the parameters. Essentially, his procedure yields a set of constants, *Ka*, *Kd*, *A*, and *B* which are used to determine γ and Δt by subsequent computations. The third procedure, proposed by Wiegand and Sanders, a modification of an iterative least squares by Deming (7), also simultaneously searches for a set of constants and should lead to a best fit. Although the time axis intercept can be computed by the Wiegand and Sanders procedures, their published data are for drug administered in solution with immediate application of first-order processes assumed. If, instead of this zero-time assumption, an intercept is computed by the delta search program, an 8-min. value is obtained with a substantially larger *Ka* providing the best fit. The suitability of assuming a zero-time intercept and essentially forcing a curve through the origin leads to other areas of importance: selection of data time points, limitations of curve extrapolation, and interpretation of computed rate constants.

Data Point Selection—Curve fitting is undertaken to obtain estimated rate constants which offer an adequate description of drug activity. It is, therefore, vital that the period of activity be sufficiently defined if the resulting constants are to be meaningful.

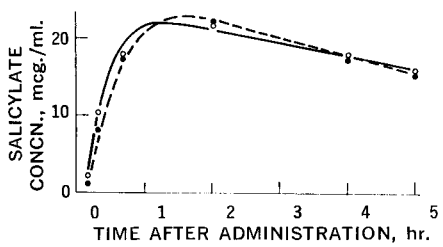

Figure 3—*Computed biexponential curves with minimized* $\Sigma(ln\ C' - ln\ C)^2$ *for 650-mg. (10-gr.) dose of aspirin. Solid line for fit to averages:* Ka = *2.62*, Kd = *0.107*, γ = *25.3*, *and* Δt = *8.0. Broken line for fit to medians:* Ka = *1.68*, Kd = *0.160*, γ = *29.4*, *and* Δt = *8.5.*

Selection of time periods, obviously, depends upon the characteristic curve for the drug. At least two or three time periods on the ascending and descending portion of the curve and one point near the peak should be selected. To establish the ascending portion accurately, the first observation should be taken as early as possible. Likewise, the descending portion should be clearly determined by later time points. Early observations are extremely critical for rapidly absorbed drugs characterized by a sharp early rise in concentration. Failure to obtain an early observation often leads to an inaccurate computed value for *Ka*. Under these circumstances, *Ka* will not be representative, and extrapolation of the curve at early moments will be misleading. Because of the mutual dependence of the constants, inadequate description of any portion of the curve can impair the accuracy of all constants.

Table I provides an example of the effect of inadequately defining the early portion of the curve. A best fit was determined for average salicylate levels from 650 mg. (10 gr.) of a buffered aspirin measured at 10, 20, 40, 120, 240, and 300 min. A best fit for the same data without the 10-min. observed average reduced the computed *Ka* from 5.61 to 4.91; in this case *Kd* and γ were not appreciably changed. The observed 10-min. concentration was 5.88 mcg./ml., and the computed 10-min. level for the fit to all points was 5.87 mcg./ml. Removing the 10-min. reading and extrapolating the computed curve result in a 10-min. estimated value of 7.95 mcg./ml., a 35% deviation from the observed value.

The descriptive quality of computed constants cannot be separated from the careful selection of data points. Drug description by pharmacokinetic rate constants is applicable only over the period observed. Therefore, the activity period of interest must be adequately covered by the observed data.

Medians and Means—To evaluate differences between fitting the model to medians and to means, a set of constants was determined for each. Salicylate levels for 18 subjects on a 650-mg. (10-gr.) dose of buffered aspirin and two separate trials of a 650-mg. (10-gr.) dose of commercial aspirin were used.

The choice of averages or medians is highly dependent upon the data. Averages are more stable and, when symmetrical distributions of concentrations are observed, deserve preference. Frequently, however, physiological characteristics of individuals are such that salicylate concentrations at early time periods fall in nonsymmetrical distributions. A few rapid drug absorbers produce averages considerably higher than the medians. Figure 3 illustrates this situation using salicylate concentrations for a 650-mg. (10-gr.) dose of aspirin. The best fit was obtained using the delta search. The fit to medians produced a lower *Ka* and a higher *Kd* than the fit to averages. Because of the skewness of the concentration distributions at early time periods, choice of the medians in this case might be desirable, since it, by definition, represents the middle value.

Table II gives the calculated constants and residual sum of squares for the best fit for the three sets of data. A comparison of sums of squares indicates that two of these three fits to averages were better than the corresponding fits to medians. This obviously does *not* imply that averages should be chosen inasmuch as the most representative statistic will not necessarily provide the lowest residual sum of squares.

Weighting of Data Points—Weighting of data points is often suggested in pharmacokinetic publications, but weighting factors are rarely proposed. Actually, the two definitions of best fit mentioned earlier represent forms of weighting. The best fit definition minimizing the sum of squared deviations appears to give equal weight to all points, but, to a degree, it is really giving more weight to the larger data values. It assumes that the reliability of all points, on the basis of absolute error, is the same. Since from an analytical point of view it is probably more accurate to assume that the reliability of all points is the same with respect to percentage error, minimizing the total squared deviations would give more weight to the larger observed values (*i.e.*, the middle portion of the curve). In contrast, minimizing the sum of squared differences in logarithms, under the assumption of equal absolute error, would give more weight to the lower observed values (*i.e.*, the ends of the curve). The choice of definition of best fit depends upon which viewpoint is more appropriate. Fitting to minimize sum of squares of differences gives a curve with greater percentage deviations of observed and computed concentrations for the low values. Minimizing squared differences in logarithms, on the other hand, presents a curve with a more uniform allocation of percent differ-

Table II—Best Fit Kinetic Constants and Residual Sum of Squares [$\Sigma(\ln C' - \ln C)^2$]

	Fit to Averages					Fit to Medians				
	Ka	*Kd*	γ	Δt	*SS*	*Ka*	*Kd*	γ	Δt	*SS*
Buffered aspirin, 650 mg. (10 gr.)	5.61	0.108	28.3	7.5	0.001596	5.45	0.110	30.0	8.5	0.005022
Aspirin, 650 mg. (10 gr.)	2.62	0.127	27.3	6.5	0.004740	1.48	0.167	30.7	6.0	0.051946
Aspirin, 650 mg. (10 gr.)	2.62	0.107	25.3	8.0	0.005381	1.68	0.160	29.4	8.5	0.001439

Table III—Comparison of Rate Constants for Best Fit

	Minimizing $\Sigma(C' - C)^2$				Minimizing $\Sigma(\ln C' - \ln C)^2$			
	Ka	*Kd*	γ	Δt, min.	*Ka*	*Kd*	γ	Δt, min.
Wagner data (tetracycline)	0.72	0.149	2.65	25.3	0.87	0.132	1.92	27.5
Lowenthal and Vitsky data (aspirin formulation D)	2.07	0.246	63.41	0	1.30	0.452	47.18	0
					4.36	0.130	53.90	6.0 (Best fit)
Wiegand and Sanders data (HT 1479)	2.30	0.254	7.70	0	2.40	0.245	7.54	0
					3.26	0.237	7.30	8.0 (Best fit)

ences of observed and computed values. Table III shows the results of fitting for each definition of best fit using data from each of the previously mentioned publications.

When choosing a definition of best fit or weighting factors, variation in the physiological characteristics among individuals should not be confused with the reliability of analytical determinations. Greater variation at early time periods of the concentration curve does not necessarily mean that the analytical accuracy is poorer at these time periods. The inaccuracy of *Ka* estimates may be partially a result of fitting for minimal sum of squared deviations or the lack of an early observation, since giving less weight to the lower observations or the lack of an early point could create greater error in estimating this portion of the curve. Minimizing sum of squared differences in logarithms should give more validity to the fit at the early and late time periods.

Other Alternatives—Using individuals or the geometric mean can present a problem for the delta search method if any observations at the early time period give a zero concentration level. If no zero levels are observed, fitting to the geometric means is equivalent to fitting data groups for individuals when minimizing the sum of squares of differences in natural logs. Obtaining a set of constants for each subject also presents fitting problems when all individual curves do not adequately fit the model. Due to the mutual dependency of a set of constants, the appropriateness of separately obtaining an average or median *Ka*, *Kd*, γ, and Δt from the group of individual sets must also be considered.

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