An Alternative Method for the Estimation of the Terminal Slope When a Few Data Points Are Available

ARISTIDES DOKOUMETZIDIS,[†] ATHANASSIOS ILIADIS,[‡] AND PANOS MACHERAS*,[§]

Contribution from *Department of Physics, University of Athens, Athens, Greece, Department of Pharmacokinetics, University of Mediterranee, Marseilles, France, and Department of Pharmacy, University of Athens, Panepistimiopolis, 15771 Athens, Greece.*

Received August 3, 1998. Final revised manuscript received November 24, 1998.
Accepted for publication January 19, 1999.

Abstract □ Phase plane plots are graphical expressions for differential equations ploting the state derivative d*c*/d*t* versus the state *c*. Using these plots, we developed a novel method for the estimation of the terminal slope from time–concentration data. The values of the derivatives used for the construction of the phase plane plots were calculated by two different methods of numerical differentiation. The first method (D1) is based on the classical calculation of slope of the line connecting two successive data points. The alternative method (D2) relies on an initial second-order polynomial interpolation utilizing three successive data points followed by the calculation of the derivative at each one of the concentration values. A forced-through-zero linear regression of the phase plane plot data is used to derive an estimate for the slope. For comparative purposes, the standard approach based on the semilogarithmic plot was also applied. For a hypothetical drug absorbed by first-order process into a one-compartment model, simulated time–concentration data disturbed by a Gaussian zero mean random error with various coefficients of variation were generated. Various sampling schedules, with two, three, four, or five data points, were utilized for the estimation of the terminal slope. Performances of the proposed methods on simulated data were expressed by means of root-mean-squared error, bias, and standard deviation. In all cases, D2 was superior to D1. The D2 method outperforms the standard method in that it furnishes estimates closer to the real values in all cases when two data points and in most cases when three data points were used. All methods behave similarly when four or five data points were used.

Introduction

The estimation of the terminal slope in time (*t*)–concentration (*c*) data can play an important role in pharmacokinetic, bioavailability, and bioequivalence studies. Routinely, this estimation is accomplished on the semilogarithmic plot of *c* vs *t*, where the slope is determined by the best line fitting the data. In some cases, two or three data points have been used to derive an estimate for the terminal slope.[1–6] Although the use of two data points to estimate terminal slope by log-linear regression is not statistically or theoretically justified, the method can be quite satisfactory when limited experimental error is encountered. However, a poor estimate may be obtained when the experimental error is high because measurements in the proximity of the least quantifiable concentration have been carried out and/or sampling has not been continued for a long enough time to ensure that absorption or disposition processes do not disturb the elimination process.

In the present study, a novel method is proposed for the estimation of the terminal slope. The method relies on the phase plane plot, which has been used for the analysis of various kinetic phenomena.[7–10] Moreover, phase plane plots of dye-dilution curves have been used to determine the cardiac output.[11] Also, this method was utilized recently for the discernment of absorption kinetics.[12]

Theoretical Section

In our previous study,[12] it was proven that for drugs obeying one-compartment model disposition and irrespective of the absorption kinetics, the slope of the terminal segment of the phase plane plot (d*c*/d*t* vs *c*) corresponds to the elimination rate constant. Similarly, when the computation was applied to a two-compartment model, this slope was equal to the smaller hybrid rate constant.[12] This observation applies to drugs following multiexponential disposition; for example

$$c(t) = \sum_{i=1}^{z} A_i e^{-\lambda_i t} \tag{1}$$

where A_i are fictitious constants in concentration units and λ_i ($i = 1, ..., z$) are hybrid rate constants ($\lambda_1 > \lambda_2 > ... > \lambda_z$). As time goes toward infinity, all but the terminal exponential term in eq 1 vanish and the latter is reduced to

$$c(t) = A_z e^{-\lambda_z t} \tag{2}$$

Taking the derivative of eq 2, we have

$$\frac{dc(t)}{dt} = -\lambda_z A_z e^{-\lambda_z t} = -\lambda_z c(t) \tag{3}$$

where λ_z is the terminal slope, which plays an important role in kinetic data analysis for determining the terminal half-life.

The last equation indicates that the negative slope of the last segment of the phase plane curve (d*c*/d*t* vs *c*) corresponds to the terminal slope. Obviously, the final state of the system ($t \rightarrow \infty$), when all the drug has been eliminated, corresponds to $c = 0$ and $dc/dt = 0$ [i.e., the last point of the phase plane is always the point (0,0)]. These observations encouraged us to examine the potential of estimating the terminal slope using the terminal limb of the phase plane curve and taking into consideration the last point (0,0), which is exact (Figure 1).

Methods

To illustrate feasibility of the proposed method and to evaluate its performances with respect to other computational approaches, a simulation study was undertaken. To this end, the one-

* To whom correspondence should be addressed. Phone: +30-1-7274675-8. Fax: +30-1-7244191. E-mail: macheras@pharm.uoa.gr.
[†] Department of Physics.
[‡] Department of Pharmacokinetics.
[§] Department of Pharmacy.

10.1021/js980317l CCC: $18.00 Published on Web 04/03/1999

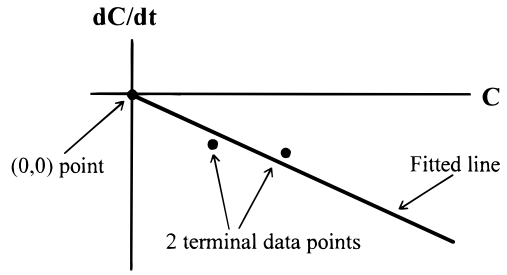

Figure 1—Schematic representation of the estimation of the terminal slope using the phase plane plot. In this example, two data points are used; the regression line is forced through (0,0), which is not an actual data point but a theoretical asymptotic limit for infinite time. The slope of the line is equal to $-k_e$.

compartment model was used associated with first-order absorption process. At time t, concentrations $c(t)$ are given by the following equation:

$$c(t) = \frac{fD}{V} \cdot \frac{k_a}{k_a - k_e} \cdot (e^{-k_e t} - e^{-k_a t}) \quad (4)$$

For normalization, the coefficient $[f \cdot D/V]$ is set to unity. The parameters k_a and k_e are absorption and elimination rate constants, respectively. Equation 4 was used to simulate concentrations in the following 5-factor controlled experimental protocol.

1. Model Parameters—One k_e value was set to reflect a 7 h half-life. Two k_a values set to reflect 1.7 and 4.6 h half-lives. Thus, the elimination rate constant was set to $k_e = 0.1\ h^{-1}$ while low and high $[k_a/k_e]$ ratios, equal to 1.5 and 4, respectively, were used.

2. Sampling Schedules—Short and long sampling schedules were examined [{6, 8, 12, 16, 20, 24} and {16, 20, 24, 28, 32, 36} h]; that is, the sampling lasts for 3.4 and 5.1 half-lives, respectively.

3. Measurement Error—Using eq 4, concentrations were computed at the sampling times and then they were corrupted by a Gaussian random zero mean measurement error. The error was variable with 5 levels of coefficient of variation (CV) at 10, 20, 30, 40, and 50%.

4. Computational Methods—The phase plane plots were constructed using two alternative methods of numerical differentiation. The first method (D1) is based on the classical calculation of slope of the line connecting two successive data points. This slope corresponds to the derivative for the mid-time point of the two data points considered. The alternative method (D2) is based initially on a second-order polynomial interpolation utilizing three successive data points. Further, the derivative is calculated at each one of the three time points considered. The method D2 as well as its application to the present study are described in the Appendix. Finally, a forced-through-zero linear regression analysis utilizing two, three, four, or five (dc/dt, c) data points is performed. In other words, a line of the form $y = ax$ is used for the fitting; the slope of this line provides an estimate for $-\lambda_z$ or for $-k_e$ for the model considered in this study. For comparative purposes, the data were also analyzed by the standard method (S) using linear regression on the semilogarithmic plot.

5. Data Analysis—Separate analyses were carried out utilizing either the last two, three, four, or five data points for both sampling schedules examined.

For each combination of the first two controlled factors (model parameters and sampling schedule) and at each one of the 5 levels of CV, 1000 measurements errors were randomly obtained and used to disturb the error-free simulated concentrations. Because of the high range of CV used, simulated data leading to positive slopes were excluded from the calculations for all methods studied. However, additional simulations were carried out to ensure a total of 1000 runs with negative slopes in all cases examined.

Simulations were done with Mathematica. The results of this simulation study were evaluated statistically. The root-mean-square error (RMSE), which provides the spread of the measures around the theoretical value ($0.1\ h^{-1}$) of the terminal slope, was calculated. Further, RMSE was split into its two components, namely, (i) the standard deviation (std), which evaluates the spread of the measures around their average value, and (ii) the bias, which provides the difference of the average of the measures from the theoretical value.

Results

For various combinations of the controlled factors utilizing either two or three data points (Figure 2) shows the results for RMSE, std, and bias. All results were derived only from data with negative slopes. The number of simulations leading to data with positive slopes increased as the level of the % CV increased for all combinations of the rest-controlled factors. Overall, the range of the percentages of data with positive slopes was 0−1.4, 0−15.2, 1−32.1, 5.6−42.8, and 18.5−57.0% for 10, 20, 30, 40, and 50% CV, respectively.

When two data points were utilized and irrespective of the CV levels, D2 yielded the lower RMSE and std values; in contrast, S yielded the higher RMSE and std values (Figure 2). In general, the higher the CV levels, the lower the performances of the methods in terms of RMSE and std. As far as the bias is concerned, D2 is the less dependent method on the CV levels.

When three data points were utilized, the performances of the methods were less dependent on the CV level (Figure 2). Moreover, in both sampling schedules, D2 method performs slightly better.

When four or five data points were utilized, the performances (not shown) of the methods were again less dependent on the CV level. All methods exhibit similar performance in terms of RMSE, std, and bias for all scenarios examined.

Discussion

The D1 and D2 methods based on the phase plane plot were developed for the estimation of the terminal slope. These methods were evaluated statistically and compared with the standard approach (S).

Two important arguments in support of the proposed D1 and D2 methods can be pointed out. First, the evaluation of the terminal slope in both methods relies on the slope of the "real terminal" segment of the phase plane curve irrespective of the sampling design applied. Second, the slope of this segment is drastically influenced by the theoretical (0,0) datum point; that is, the forced-through-zero linear regression (Figure 1).

When two data points were used, the proposed methods (D1 and especially D2) perform better than the S approach for all combinations of controlled factors examined. In particular, the D2 method had remarkably smaller RMSE, std, and bias than the D1 and S methods. The superiority of the D1 and D2 methods with respect to the S approach should be attributed to the considerable contribution of the exact datum point (0,0) in determining the slope of the regression line in the phase plane plot. In fact, the regression line (i) passes through this errorless datum point and (ii) satisfies the least squares condition for the other two (dc/dt, c) data points. These observations should be considered in conjunction with the low std and the negligible bias of the D2 method when applied to the long sampling interval conditions. Hence, the best performance of the D2 method is noted when two data points near the end of the sampling schedule are used. The poorer characteristics of the D1 method in comparison with the D2 method are most likely associated with the refined methodology used for the calculation of the derivatives in the D2 method. In addition, the D2 method utilizes the same number, whereas the D1 method utilizes one less of the data points (dc/dt, c) than the available (c, t) data points.

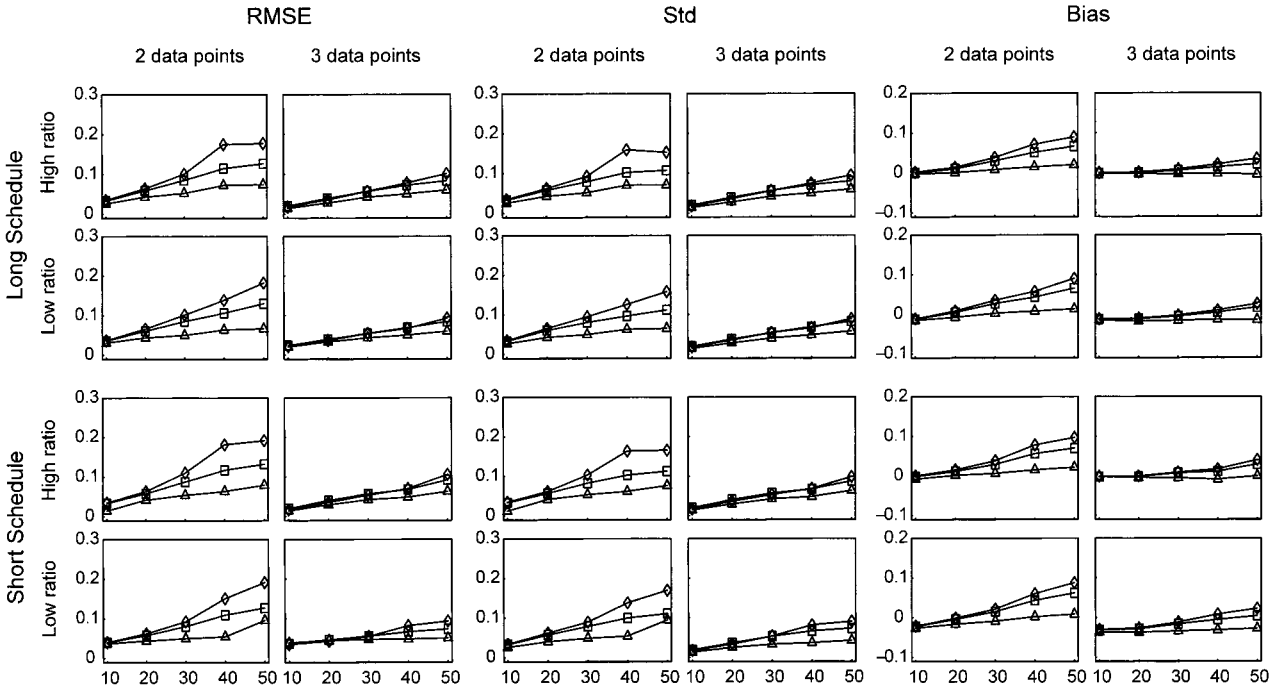

Figure 2—For all combinations of the controlled factors and for the three methods S, D1, and D2, utilizing either two or three data points, the RMSE, the std, and the bias are shown as a function of CV of measurement errors. Key: (◇) method S; (□) method D1; (△) method D2.

The D2 method draws power from the computational method that adds another data point at 0,0. Clearly, this power is more dramatic with few data points.

When three data points were used, the D2 method is marginally superior to the others for both sampling schedules. Methods D1 and S exhibit higher dependence on the CV levels for all parameters studied (Figure 2). This higher dependence is so despite the diminished contribution of the errorless datum point (0,0), which is now one out of four data points in determining the slope. However, for the worst scenario (short schedule-low ratio of rate constants), the D2 method underestimates the true value of k_e (Figure 2).

We recently used the phase plane approach jointly with the D1 method in investigating the absorption kinetics.[12] The results of the present study comparing the D1 and D2 methods in estimating the terminal slope allow someone to infer that the analytical power of the phase plane approach in investigating the absorption kinetics will be improved if the D2 method is used in place of the D1 method. Indeed, we carried out a number of simulations (results not shown), and the D2 method was superior.

For the sake of completion, one should add that the forced-through-zero classical linear regression analysis applied to the phase plane plot data does not take into account the error associated with the *x*-variable (*c* in abscissa). Special procedures are used in the literature in those situations where both variables are subject to error.[13,14] To this end, we applied the D2 method to our simulated data utilizing the geometric mean functional relationship (GMFR)[14] approach to linear regression for fitting. For the same sets of data, the GMFR approach did not provide better estimates for k_e when compared with the estimates derived from the classical linear regression. It seems likely that the error associated with the *y*-variable (d*c*/d*t* in ordinate) is considerably higher than the corresponding for *x*-variable (concentration *c*) and, therefore, the GMFR approach does not offer any advantage over the classical regression for the proper analysis of data.

Conclusions

The phase plane methods D1 and D2 perform better than the semilogarithmic approach for all scenarios examined when two or three data points are used for the estimation of the terminal slope. When four or five data points are available, all methods, D1, D2, and S, behave similarly.

The D2 method described here appears to be a robust approach to provide a reliable estimate for the terminal slope when two or three data points are available. Although essentially new, the D2 method can be handled computationally by simple calculations of the derivative at the various time points using interpolation techniques, such as spline functions, Lagrange polynomials, etc.[15] It should be emphasized that the technique of Lagrange polynomials used here provides a unique polynomial for a given (*c*,*t*) data set and, therefore, the method developed is not user dependent.

Appendix

The D2 method relies on the general problem of finding the Lagrange interpolating polynomial,[15] which is equal to the function describing the time course of drug in the body at a certain number of specified time points; that is, the experimental data points (c_j, t_j). We use three successive data points ($j = 1, 2, 3$) and derive the unique quadratic polynomial $c = pt^2 + qt + h$, which passes through these points.

The polynomial coefficients *p*, *q*, and *h* can be calculated by solving the following system:

$$\left.\begin{aligned} pt_1^2 + qt_1 + h &= c_1 \\ pt_2^2 + qt_2 + h &= c_2 \\ pt_3^2 + qt_3 + h &= c_3 \end{aligned}\right\} \qquad \text{(A1)}$$

Furthermore, the derivative dc_j/dt at any of these three points c_j is given by

$$\frac{dc_j}{dt} = 2pt_j + q \qquad j = 1, 2, 3 \tag{A2}$$

Solving the system A1 and replacing in A2 we obtain the following required derivatives:

$$\frac{dc_j}{dt} = c_1\left[\frac{2t_j - t_2 - t_3}{(t_1 - t_2)(t_1 - t_3)}\right] + c_2\left[\frac{2t_j - t_1 - t_3}{(t_2 - t_1)(t_2 - t_3)}\right] + c_3\left[\frac{2t_j - t_1 - t_2}{(t_3 - t_1)(t_3 - t_2)}\right] \tag{A3}$$

for every middle point of the data set $j = 2$. For the first and the last data points of the set that are not surrounded by other points, j is taken to be 1 and 3, respectively.

It is well-known that the derivative is more accurately calculated at the middle point.[15] In the present study, we took advantage of this fact and calculated all the derivatives of the data points used for the estimation of k_e by applying $j = 2$ in eq A3; for example, for the sampling schedule {6, 8, 12, 16, 20, 24} h, the derivatives at 8, 12, 16, and 20 h were calculated from the triplets (6, 8, 12), (8, 12, 16), (12, 16, 20), and (16, 20, 24) h, respectively. Unavoidably, the derivatives at the final points were calculated with eq A3 using $j = 3$ [e.g., for the sampling schedule just mentioned, the derivative at 24 h was calculated from the triplet (16, 20, 24) h].

References and Notes

1. Hamaguchi, T.; Shinkuna, D.; Yamanaka, Y.; Mitsuno, N. Bioavailability of mefenamic acid: influence of food and water intake. *J. Pharm. Sci.* **1986**, *75*, 891−893.
2. Sanders, S. N.; Michaelis, K.; Maurette, J. M.; Jaeger, H. Relative bioavailability of two spray formulations of nitroglycerin. *J. Pharm. Sci.* **1986**, *75*, 244−246.
3. Macheras, P.; Reppas, C. Studies on drug-milk freeze-dried formulations I: Bioavailability of sulfamethizole and dicumarol formulations. *J. Pharm. Sci.* **1986**, *75*, 692−696.
4. Midha, K. K.; Chakraborty, R.; Schwede, E. M.; Hawes, G.; McKay, J. W.; Hubbard, J. K.; Cooper, M.; Moore, M. Comparative bioavailability of two tablet formulations of phluphenazine dihydrochloride in drug-free psychiatric patients. *J. Pharm. Sci.* **1990,** *79*, 3−8.
5. Cefali, E. A.; Baufieled, C. R.; Gonzalez, M. A.; Wagner, J. G. In vivo determination of zero-order absorption from a transdermal glycerol trinitrate system. *Eur. J. Pharm. Biopharm.* **1993**, *39*, 140−143.
6. Liu, X.; Brouwer, K. L. R.; Pollack, G. M. A modified residual method to estimate the zero-order absorption rate constant in a one-compartment model. *Bioph. Drug Dispos.* **1997**, *18*, 93−101.
7. Tchernichovski, O.; Golani, I. A phase plane representation of rat exploratory behavior. *J. Neurosci. Meth.* **1995**, *62*, 21−27.
8. Cooke, J. D.; Brown, S. H. Phase plane tracking: a new method for shaping movements. *Brain Res. Bull.* **1986**, *16*, 435−437.
9. Karagueuzian, H. S.; Khan, S. S.; Denton, T. A.; Gotoh, M.; Mandel, W. J.; Diamond, G. A. Phase plane plot of electrograms as a marker of ventricular electrical instability during acute ischemia: initial experimental results and potential clinical applications. *Pacing Clin. Electrophysiol.* **1992**, *15*, 2188−2193.
10. Fonseca-Costa, A.; Magrassi, P.; Zin, W. A.; Romeo, L. J. M., Jr. Detection and quantification of small right-to-left shunts by the phase-plane method. *Am. J. Physiol.* **1984**, *247*, H517−H522.
11. Fonseca-Costa, A.; Zin, W. A. Cardiac output and mean transit time using the phase plane of dye-dilution curves. *Am. J. Physiol.* **1979**, *236*, H798−H801.
12. Dokoumetzidis, A.; Macheras, P. Investigation of absorption kinetics by the phase plane method *Pharm. Res.* **1998**, *14*, 1262−1269.
13. Seber, S. A. F.; Wild, C. J. Errors-in-variables models. In *Nonlinear Regression*; Wiley: New York, 1989; Ch.10.
14. Valsami, G.; Macheras, P. The geometric mean functional relationship approach to linear regression in pharmaceutical studies: application to the estimation of binding parameters. *Pharm. Sci.* **1995**, *1*, 551−554.
15. Burden, R. L.; Faires, J. D. *Numerical Analysis*; PWS Publishing: Boston, 1993; p 159.

Acknowledgments

Supported in part by the General Secretariat for Research and Development (PENED Grant 70/3/2824).

JS980317L