(5) S. L. Ali, *Int. J. Pharm.*, **5**, 85 (1980).
(6) D. B. Black, R. C. Lawrence, E. G. Lovering, and J. R. Watson, *J. Pharm. Sci.*, **70**, 208 (1981).
(7) J. T. Stewart and J. L. Williamson, *Anal. Chem.*, **48**, 1182 (1976).
(8) J. Troschuetz, *Arch. Pharm.*, **314**, 204 (1981).
(9) E. Jacobsen and T. V. Jacobsen, *Anal. Chim. Acta*, **55**, 293 (1971).
(10) W. Lund and L. Opheim, *Anal. Chim. Acta*, **88**, 275 (1977).
(11) H. Raber and J. Gruber, *Scientia Pharm.*, **40**, 35 (1972).
(12) Y. A. Beltagy, A. S. Issa, and M. S. Mahrous, *Egypt. J. Pharm. Sci.* **19**, 115 (1978).
(13) R. W. T. Seitzinger, *Pharm. Weekbl.*, **110**, 1073 (1975).
(14) W. N. French, F. F. Matsui, and S. J. Smith, *J. Pharm. Sci.*, **64**, 1545 (1975).
(15) T. D. Doyle and F. R. Fazzari, *J. Pharm. Sci.*, **63**, 1921 (1974).
(16) A. G. Davidson, *J. Pharm. Pharmacol.*, **28**, 795 (1976).
(17) I. Sunshine "CRC Handbook of Spectrophotometric Data of Drugs," CRC Press, Boca Raton, Fla., 1981 p. 143.
(18) J. Barrett, W. Franklyn Smyth, and I. E. Davidson, *J. Pharm. Pharmacol.*, **25**, 387 (1973).
(19) J. Barrett, W. Franklyn Smyth, and J. P. Hart, *J. Pharm. Pharmacol.*, **26**, 9 (1974).
(20) A. Albert and E. P. Serjeant "The Determination of Ionisation Constants," 2nd ed., Chapman and Hall, London, 1971, p. 44.
(21) A. L. Wilson, *Analyst*, **86**, 72 (1961).

# Initial Slope Technique for Estimation of the Apparent Volume of Distribution During Constant-Rate Intravenous Infusion

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**Abstract** □ A new technique is presented for estimating the apparent volume of distribution of drugs during constant-rate intravenous infusion. It is based on the initial slope of the plasma drug concentration *versus* time profile during the infusion. Equations are derived to provide estimates of the apparent volume of distribution for a one-compartment drug and for the central compartment of a two-compartment drug. The utility of the technique is illustrated by data obtained during constant-rate infusion of metronidazole in 11 healthy subjects. The average estimated value of the volume of the central compartment of metronidazole was 12% higher than the average value obtained by conventional pharmacokinetic analysis. The systematic error associated with this volume estimation procedure was assessed through the use of dimensionless concentration *versus* dimensionless time plots. The initial slope technique should prove useful in providing initial estimates of volume terms.

**Keyphrases** □ Pharmacokinetics—apparent volume of distribution, constant-rate infusion, estimation by an initial slope technique, application to metronidazole □ Initial slope technique—estimation of apparent volume of distribution, constant-rate infusion, application to metronidazole pharmacokinetics □ Infusion, constant-rate—estimation of apparent volume of distribution, initial slope technique, application to metronidazole pharmacokinetics

During the past few years, three research groups have described approaches for determining the apparent volume of distribution after administration of single and multiple intermittent, constant-rate, intravenous infusions. Sawchuk *et al.* (1, 2) derived an equation from which the apparent volume of distribution for a drug that follows a one-compartment open model can be calculated, based on knowledge of (*a*) the elimination rate constant, (*b*) the preinfusion residual plasma drug concentration, and (*c*) the plasma drug concentration at the end of infusion. Chiou *et al.* (3, 4) extended the technique by using post-infusion data and the midpoint back-extrapolation method to calculate the apparent volume of distribution for drugs that exhibit linear one-compartmental or multicompartmental characteristics. In addition, application of the Chiou–Hsu equation (5–7) to accurately estimate total body clearance during a constant-rate intravenous infusion requires an accurate estimate of the apparent volume of distribution. Barzegar-Jalali (8) used equally spaced sampling times during a zero-order intravenous infusion and the first derivative of the plasma drug concentration *versus* time profile to directly estimate both the elimination rate constant and steady-state plasma drug concentration for a drug with linear one-compartmental characteristics. The apparent volume of distribution could then be estimated from the latter two quantities.

Unfortunately, none of these approaches can be used to estimate directly the apparent volume of distribution or total body clearance from individual patient plasma drug concentration data gathered during the ongoing infusion. The purpose of this paper is to detail a method for estimating the apparent volume of distribution of a drug from the initial slope of the plasma drug concentration *versus* time profile during a constant-rate intravenous infusion.

## THEORETICAL

If instantaneous drug distribution and first-order drug elimination are assumed, the plasma concentration of a drug given as a constant-rate intravenous infusion can be described by the following equation (9):

$$C = \frac{k_0}{KV}(1 - e^{-Kt}) \qquad \text{(Eq. 1)}$$

where $C$ is concentration, $k_0$ is the zero-order infusion rate, $K$ is the first-order elimination rate constant, $V$ is the apparent volume of distribution, and $t$ is time. Taking the first derivative of Eq. 1 with respect to time yields:

$$\frac{dC}{dt} = \frac{k_0 e^{-Kt}}{V} \qquad \text{(Eq. 2)}$$



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which can be evaluated at time zero to give:

$$\frac{dC}{dt}(t=0) = \frac{k_0}{V} \qquad \text{(Eq. 3)}$$

Rearrangement of Eq. 3 yields:

$$V = \frac{k_0}{\frac{dC}{dt}(t=0)} \qquad \text{(Eq. 4)}$$

Equation 4 indicates that the apparent volume of distribution can be calculated from the zero-order infusion rate and the initial slope of the plasma drug concentration *versus* time curve.

The above mathematical manipulations are readily extended to a drug undergoing biexponential disposition following drug administration by constant-rate intravenous infusion. Plasma drug concentration during infusion is given by (10):

$$C = \frac{k_0}{k_{10}V_c}\left[1 - \left(\frac{k_{10}-\beta}{\alpha-\beta}\right)e^{-\alpha t} - \left(\frac{\alpha-k_{10}}{\alpha-\beta}\right)e^{-\beta t}\right] \qquad \text{(Eq. 5)}$$

where $\alpha$ is the rapid disposition rate constant, $\beta$ is the slow disposition rate constant, $k_{10}$ is the first-order elimination rate constant from the central compartment, and $V_c$ is the apparent volume of distribution of the central compartment. Taking the first derivative with respect to time yields:

$$\frac{dC}{dt} = \frac{\alpha k_0(k_{10}-\beta)}{k_{10}V_c(\alpha-\beta)}e^{-\alpha t} + \frac{\beta k_0(\alpha-k_{10})}{k_{10}V_c(\alpha-\beta)}e^{-\beta t} \qquad \text{(Eq. 6)}$$

which can be evaluated at time zero to give:

$$\frac{dC}{dt}(t=0) = \frac{k_0}{V_c} \qquad \text{(Eq. 7)}$$

Rearrangement of Eq. 7 yields:

$$V_c = \frac{k_0}{\frac{dC}{dt}(t=0)} \qquad \text{(Eq. 8)}$$

Equation 8 shows that, for a drug exhibiting two-compartmental characteristics, the apparent volume of distribution of the central compartment is equal to the quotient of the zero-order infusion rate and the initial slope of the plasma drug concentration *versus* time curve. Equations 4 and 8 indicate that the apparent volume of distribution can be estimated if the initial slope of the concentration *versus* time profile can be estimated. This initial slope can be estimated by:

$$\frac{dC}{dt}(t=0) \cong \frac{\Delta C}{\Delta t}(t \to 0) \qquad \text{(Eq. 9)}$$

which is equivalent to:

$$\frac{dC}{dt}(t=0) \cong \frac{C}{t} \qquad \text{(Eq. 10)}$$

when there is no residual drug concentration at time zero. Using this simple estimate of the initial slope, Eqs. 4 and 8 become:

$$V^{est} = \frac{k_0}{C/t} \qquad \text{(Eq. 4a)}$$

and

$$V_c^{est} = \frac{k_0}{C/t} \qquad \text{(Eq. 8a)}$$

where the superscript est indicates an estimated value of the volume term.

A detailed examination of the concentration *versus* time profiles for both the monoexponential and biexponential disposition cases is necessary to determine the systematic error associated with this volume estimation procedure. To make this error analysis generally applicable to drugs with a wide range of parameter values, it is advantageous to work with the concentration *versus* time equations in their dimensionless forms. The dimensionless equivalent of Eq. 1 is:

$$\frac{C}{C_{ss}} = 1 - e^{-\phi} \qquad \text{(Eq. 11)}$$

where $\phi \equiv Kt$ and $C_{ss} = (k_0/KV)$, *i.e.*, $C$ at steady state. Equation 11 shows that the dimensionless concentration $C/C_{ss}$ is a function only of the dimensionless time $\phi$ and therefore Eq. 11 is applicable to any drug undergoing monoexponential disposition. The dimensionless equivalent of Eq. 5 is:

$$\frac{C}{C_{ss}} = 1 - \left(\frac{1-\rho_2}{\rho_1-\rho_2}\right)e^{-\rho_1\phi} - \left(\frac{\rho_1-1}{\rho_1-\rho_2}\right)e^{-\rho_2\phi} \qquad \text{(Eq. 12)}$$

where $\phi \equiv k_{10}t$, $\rho_1 = \alpha/k_{10}$, $\rho_2 = \beta/k_{10}$, and $C_{ss} = (k_0/k_{10}V_c)$, *i.e.*, $C$ at steady state. In concert with the classical definition for a conventional biexponential disposition model where $\alpha > k_{10} > \beta$, it follows that $\rho_1 > 1 > \rho_2$. In contrast to Eq. 11, Eq. 12 indicates that the dimensionless concentration of a two-compartmental drug is a function not only of the dimensionless time, but also of the dimensionless hybrid rate constants $\rho_1$ and $\rho_2$.

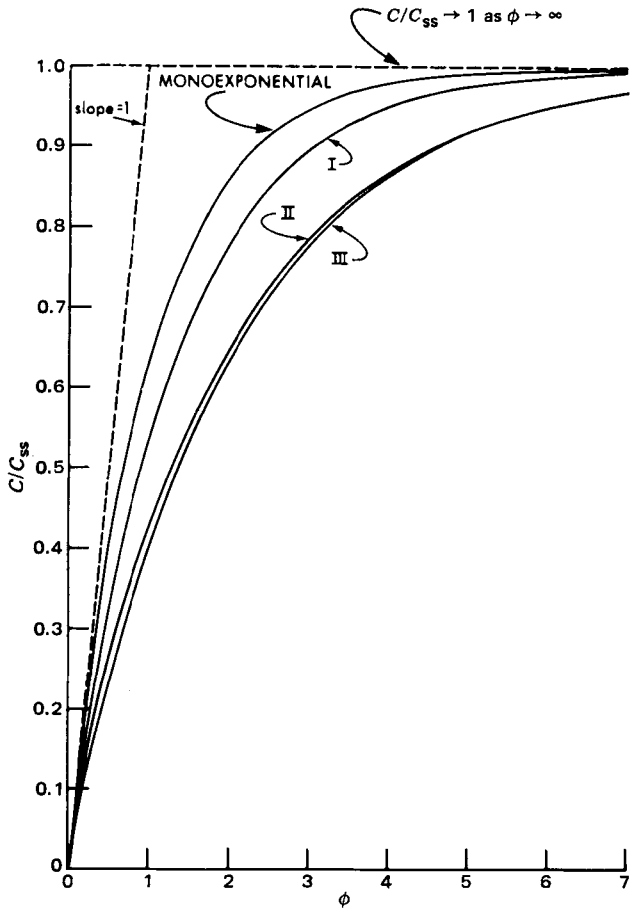


**Figure 1**—*Relationship between dimensionless concentration* $C/C_{ss}$ *and dimensionless time* $\phi$ *for the universal monoexponential disposition model (Eq. 11) and three examples (I, II, and III) of the biexponential disposition model (Eq. 12). Parameter values were chosen to reflect the range of values observed for the metronidazole examples. Key: I:* $\rho_1 = 10$, $\rho_2 = 0.75$; *II:* $\rho_1 = 10$, $\rho_2 = 0.50$; *III:* $\rho_1 = 30$, $\rho_2 = 0.50$.

The utility of the dimensionless Eqs. 11 and 12 is illustrated in Fig. 1. The most striking feature of these curves is that they share common asymptotes at both $\phi = 0$ and $\phi = \infty$. In each case, the initial slope is unity (as indicated by the dashed line in Fig. 1). This is readily verified by differentiation of Eqs. 11 and 12 and evaluation of the respective derivatives at $\phi = 0$. Note that the unit initial slope feature for the biexponential disposition model is preserved only when the dimensionless parameters $\phi$, $\rho_1$, and $\rho_2$ are defined as above. The family of exemplary curves for the biexponential model in Fig. 1 indicates that $\rho_1$ and $\rho_2$ are useful indices of the degree of biexponential character, *i.e.*, the extent of deviation from the monoexponential model. For a given value of $\rho_2$, the degree of biexponential character increases with increasing values of $\rho_1$, thereby reflecting the divergence of $\alpha$ and $\beta$. As $\rho_2$ increases (for a given value of $\rho_1$), the biexponential model exhibits less pronounced biexponential character and collapses to the monoexponential model as $\rho_2$ approaches 1, *i.e.*, as $\beta$ becomes experimentally indistinguishable from $k_{10}$. Thus, the dimensionless plot (Fig. 1) affords inspection of both monoexponential and biexponential disposition cases under limiting conditions ($\phi \to 0$, $\phi \to \infty$) that supply equal asymptotes and elucidates the conditions under which collapse to the monoexponential case occurs.

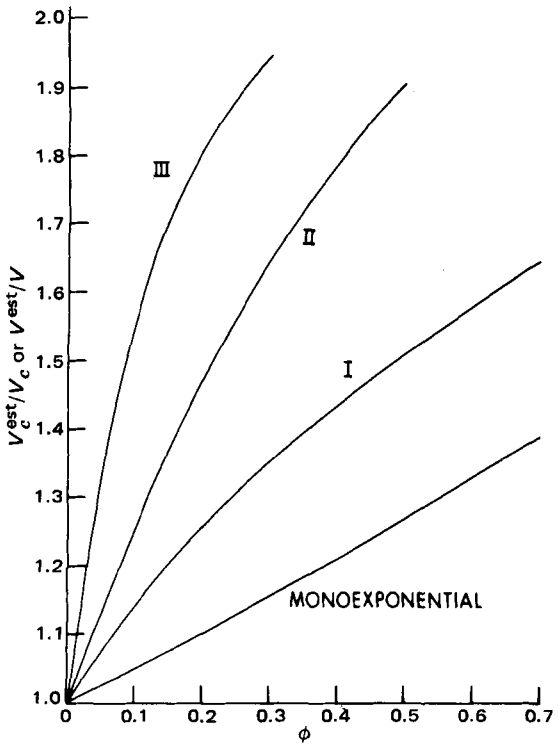


**Figure 2**—*Relationship between the ratio of estimated volume to true volume* versus *dimensionless time $\phi$ for the universal monoexponential disposition model (Eq. 16) and three examples (I, II, and III) of the biexponential disposition model (Eq. 19). The biexponential model cases are defined in Fig. 1.*

These features are useful in evaluation of the errors associated with volume estimation.

A convenient way to quantify the accuracy of the initial slope technique for volume estimation is to examine the ratio of the estimated volume to the true volume. Combination of Eqs. 4 and 4a yields:

$$\frac{V^{est}}{V} = \frac{\frac{dC}{dt}(t=0)}{C/t} \qquad \text{(Eq. 13)}$$

or

$$\frac{V^{est}}{V} = \frac{\frac{d(C/C_{ss})}{d\phi}[\phi = 0]}{\frac{(C/C_{ss})}{\phi}} \qquad \text{(Eq. 14)}$$

Recognize that the numerator in Eq. 14 is simply the unit initial slope of the previously developed dimensionless plot (Fig. 1). Therefore, Eq. 14 reduces to:

$$\frac{V^{est}}{V} = \frac{\phi}{C/C_{ss}} \qquad \text{(Eq. 15)}$$

which on substitution of Eq. 11 yields:

$$\frac{V^{est}}{V} = \frac{\phi}{1 - e^{-\phi}} \qquad \text{(Eq. 16)}$$

Equation 16 shows that the accuracy of the volume estimate is a function only of the dimensionless sampling time $\phi$. In an analogous fashion for the biexponential model, Eqs. 8 and 8a yield:

$$\frac{V_c^{est}}{V_c} = \frac{\frac{dC}{dt}(t=0)}{C/t} \qquad \text{(Eq. 17)}$$

or

$$\frac{V_c^{est}}{V_c} = \frac{\frac{d(C/C_{ss})}{d\phi}[\phi = 0]}{\frac{(C/C_{ss})}{\phi}} \qquad \text{(Eq. 18)}$$

**Table I—Calculated Sampling Time to Achieve an Acceptable Accuracy of Volume Estimation for Drugs Undergoing Monoexponential Disposition with Various Half-lives**

| Acceptable Accuracy, $V^{est}/V$ | Maximum Allowable Value of $\phi$[a] | $t_{1/2}$, h | Corresponding Maximum Allowable Intrainfusion Sampling Time ($t$), min[b] |
|---|---|---|---|
| 1.01 | 0.020 | 2 | 3.5 |
| | | 4 | 6.9 |
| | | 8 | 13.9 |
| | | 12 | 20.8 |
| | | 24 | 41.6 |
| 1.02 | 0.040 | 1 | 3.5 |
| | | 2 | 6.9 |
| | | 4 | 13.9 |
| | | 8 | 27.7 |
| | | 12 | 41.6 |
| | | 24 | 83.1 |
| 1.05 | 0.098 | 0.5 | 4.2 |
| | | 1 | 8.5 |
| | | 2 | 17.0 |
| | | 4 | 33.9 |
| | | 8 | 67.9 |
| | | 12 | 101.8 |
| | | 24 | 203.6 |
| 1.10 | 0.194 | 0.25 | 4.2 |
| | | 0.50 | 8.4 |
| | | 1 | 16.8 |
| | | 2 | 33.6 |
| | | 4 | 67.2 |
| | | 8 | 134.4 |
| | | 12 | 201.6 |
| | | 24 | 403.1 |

[a] Calculated *via* Eq. 16. [b] Calculated *via* Eq. 21.

Substitution of the unit initial slope and Eq. 12 leads to an expression analogous to Eq. 16:

$$\frac{V_c^{est}}{V_c} = \frac{\phi}{1 - \left(\frac{1-\rho_2}{\rho_1-\rho_2}\right)e^{-\rho_1\phi} - \left(\frac{\rho_1-1}{\rho_1-\rho_2}\right)e^{-\rho_2\phi}} \qquad \text{(Eq. 19)}$$

Equation 19 shows that the accuracy of the volume estimate is a function of $\rho_1$ and $\rho_2$, as well as the dimensionless sampling time $\phi$. The ratios of estimated volume to true volume calculated from Eqs. 16 and 19 are illustrated in Fig. 2. In Fig. 2, as in Fig. 1, the monoexponential disposition case applies to all drugs with such dispositional properties. Consistent with Fig. 1, Fig. 2 shows that the estimation error associated with the biexponential case approaches that of the monoexponential case as $\rho_2$ approaches 1. Note the tendency of the initial slope technique to overestimate the apparent volume of distribution. This is consistent with the fact that the true initial slope, $dC/dt(t = 0)$, is underestimated by $C/t$, which is the slope of a line from the origin to the point $t,C$ (see Eq. 10 and Fig. 1). This tendency to overestimate the volume term can also be readily verified by estimation of the quotient $C/t$ using Maclaurin series expansion (11) for limited term approximation of the exponential terms for concentration in Eqs. 1 and 5[1]. Clearly, accurate estimation of the apparent volume of distribution for both the monoexponential and biexponential model cases is best accomplished using a short intrainfusion dimensionless sampling time. Since this dimensionless sampling time is a function of both sampling time and the appropriate rate constant, it is useful to consider the accuracy of volume estimation in terms of sampling time ($t$) for drugs having a particular half-life ($t_{1/2}$). For the case of monoexponential disposition, $\phi \equiv Kt$ and therefore:

$$t = \frac{\phi}{K} \qquad \text{(Eq. 20)}$$

or

$$t = \frac{\phi t_{1/2}}{\ln 2} \qquad \text{(Eq. 21)}$$

Similarly, for the biexponential disposition case, $\phi \equiv k_{10}t$ and therefore:

$$t = \frac{\phi}{k_{10}} \qquad \text{(Eq. 22)}$$

[1] D. M. Cocchetto, unpublished calculations.

**Table II—Comparison of the Estimates of $V_c$ by the Initial Slope Technique and by Nonlinear Regression Analysis for 11 Subjects Receiving Metronidazole**

| Subject | Plasma Metronidazole Concentration At 15 min[a], μg/mL | $C/t$, μg/mL·min[b] | $V_c^{est}$ (1) By Initial Slope Technique[c] | $V_c$ (1) By Nonlinear Regression | $\frac{V_c^{est}}{V_c}$[d] | $\left(\frac{V_c^{est}}{V_c}\right)_{calc}$[e] |
|---|---|---|---|---|---|---|
| 1 | 12.68 | 0.845 | 31.6 | 33.3 | 0.95 | 1.07 |
| 2 | 10.88 | 0.725 | 36.8 | 32.7 | 1.13 | 1.11 |
| 3 | 14.16 | 0.944 | 28.3 | 23.2 | 1.22 | 1.22 |
| 4 | 11.16 | 0.744 | 35.8 | 26.2 | 1.37 | 1.06 |
| 5 | 12.61 | 0.841 | 31.7 | 31.1 | 1.02 | 1.10 |
| 6 | 15.52 | 1.035 | 25.8 | 18.9 | 1.37 | 1.37 |
| 7 | 14.36 | 0.957 | 27.9 | 27.4 | 1.02 | 1.15 |
| 8 | 19.57 | 1.305 | 20.4 | 21.6 | 0.94 | 1.17 |
| 9 | 13.13 | 0.875 | 30.5 | 27.5 | 1.11 | 1.04 |
| 10 | 13.75 | 0.917 | 29.1 | 26.8 | 1.09 | 1.08 |
| 11 | 10.35 | 0.690 | 38.7 | 33.8 | 1.14 | 1.02 |

[a] Each subject received a constant-rate intravenous infusion of 800 mg of metronidazole over 30 min ($k_0$ = 26.67 mg/min). [b] An estimate of $dC/dt(t = 0)$ calculated as the slope of the line joining the origin to the point (15 min, $C$). [c] Calculated *via* Eq. 8a. [d] For each subject, this quantity is the quotient of the quantities in columns 4 and 5. [e] Calculated *via* Eq. 19 using the values of $\phi$, $\rho_1$, and $\rho_2$ obtained for each subject (see footnote 2).

or

$$t = \frac{\phi t_{1/2}^{10}}{\ln 2} \qquad \text{(Eq. 23)}$$

where the superscript 10 signifies the half-life with respect to $k_{10}$. Table I summarizes the intrainfusion sampling time $t$, calculated *via* Eq. 21, required to achieve an acceptable accuracy of volume estimation for drugs undergoing monoexponential disposition with various half-lives. These results suggest that an accurate initial estimate of $V$ (error ≤ 10%) can be obtained from a single intrainfusion concentration determined at a clinically realistic sampling time for drugs with a half-life as short as 15 min. Clearly, the accuracy of volume estimation increases as half-life increases for a series of drugs studied at the same intrainfusion sampling time.

Of special interest with respect to the monoexponential disposition case is the utility of multiple intrainfusion sampling times. Each of two or more sequential intrainfusion sampling times can be used to obtain increasingly errant overestimates of $V$. In view of the approximately linear character of the monoexponential curve in Fig. 2, a plot of these $V^{est}$ values *versus* intrainfusion sampling time should be nearly linear. The time zero ordinate intercept of the $V^{est}$ *versus* intrainfusion sampling time plot then provides a more accurate value of $V^{est}$. This intercept volume estimation procedure should prove particularly useful for drugs with short half-lives (<30 min) and monoexponential disposition characteristics, since an accurate value of $V^{est}$ can be calculated from multiple values of $V^{est}$ determined at intrainfusion sampling times that are long relative to the half-life.

An analysis similar to that summarized in Table I for the monoexponential case can be done for the biexponential disposition case, but numerical values of $\rho_1$ and $\rho_2$ must first be chosen. For example, for each of the biexponential cases illustrated in Fig. 2, the accuracy of volume estimation could be assessed and tabulated as a function of the half-life ($t_{1/2}^{10}$) by applying Eq. 19 to calculate the $\phi$ value yielding an acceptable accuracy and then using Eq. 23 to calculate the necessary sampling time $t$. Since this exercise requires estimates of $\rho_1$ and $\rho_2$ for an individual patient, it is best left to the reader having specific parameters for a drug of interest. Note that estimation of $V_c$ as the time zero ordinate intercept of a plot of $V_c^{est}$ *versus* intrainfusion sampling time is not recommended, since this approach would be accurate only if this relationship were approximately linear. The biexponential curves in Fig. 2 clearly show that reasonable linearity can not be ensured over the range of possible values of $\rho_1$ and $\rho_2$ for every drug.

## RESULTS AND DISCUSSION

The utility of the initial slope technique in estimating the apparent volume of distribution of the central compartment will now be illustrated using data collected in a recent study on metronidazole administered as a constant-rate intravenous infusion. A total of 800 mg of metronidazole in solution was administered over 30 min to 11 healthy human volunteers[2]. The individual subject plasma metronidazole concentration *versus* time data were analyzed using the nonlinear least-squares regression program NONLIN (12, 13) in conjunction with a subroutine for the two-compartment open model with zero-order intravenous infusion input. For purposes of this example, the NONLIN parameter estimate of $V_c$ for each patient will be compared to $V_c^{est}$ calculated by Eq. 8a. The denominator in Eq. 8a is calculated from the plasma metronidazole concentration observed at 15 min after start of the infusion (the earliest sampling time in the study). Table II indicates the simplicity of this $V_c^{est}$ calculation. Figure 3 illustrates the correlation between values of $V_c^{est}$ and the nonlinear regression values of $V_c$.

This example demonstrates use of the initial slope technique in estimating $V_c$. Clearly, this estimate should be subsequently refined *via* more thorough characterization of the plasma drug concentration *versus* time profile. The predicted tendency of the initial slope technique of volume estimation to overestimate the true value of the volume term is confirmed by the example for metronidazole (Fig. 3, Table II) since 9 of the 11 subjects had higher initial slope estimates of $V_c$ compared with the nonlinear regression values of $V_c$; on the average, the former estimates were 12% higher. The magnitudes of the observed $V_c^{est}/V_c$ ratios (column 6 in Table II) are in reasonable agreement with the theoretical ratios of $V_c^{est}/V_c$ (column 7 in Table II) calculated *via* Eq. 19 using the individual subject values of $\phi$, $\rho_1$, and $\rho_2$. Deviation of the observed ratios from the theoretical ratios is probably due to measurement errors in blood sampling time and plasma metronidazole concentrations. Care must always be taken to minimize such measurement errors prior to using analytical results for pharmacokinetic parameter estimation.

A new technique has been presented for estimating the apparent vol-

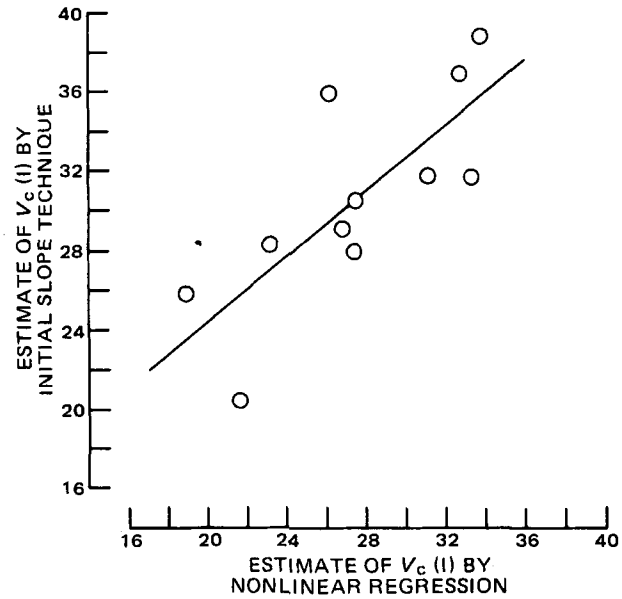


**Figure 3**—*Correlation between* $V_c$ *estimated by the initial slope technique and the nonlinear regression estimate of* $V_c$ *in 11 subjects receiving a constant-rate intravenous infusion of metronidazole. The linear regression line is shown (slope = 0.819, y-intercept = 8.09,* r = *0.768).*

[2] T. Bergan and D. M. Cocchetto, "Pharmacokinetics of Metronidazole After Intravenous, Oral, and Rectal Administration," manuscript in preparation.

ume of distribution of drugs during constant-rate intravenous infusion. This technique is based on the initial slope of the plasma drug concentration *versus* time profile during infusion. The resulting estimate of the apparent volume of distribution should prove useful as an initial estimate of this pharmacokinetic parameter, especially for investigational drugs for which no pharmacokinetic parameter estimates are available, until additional data permit estimation of the appropriate volume term by conventional methods (14). In addition, this estimate of the apparent volume of distribution can be used with two intrainfusion concentration *versus* time points as input to the Chiou–Hsu equation (5–7), from which total body clearance for individual patients can be calculated. In illustrating the theoretical principles and potential errors involved in the initial slope technique, equations were derived and plots presented of dimensionless concentration *versus* dimensionless time. To our knowledge such an approach, although widely used in engineering, has not been used previously in pharmacokinetics.

## REFERENCES


(1) R. J. Sawchuk and D. E. Zaske, *J. Pharmacokinet. Biopharm.*, **4**, 183 (1976).

(2) R. J. Sawchuk, D. E. Zaske, R. J. Cipolle, W. A. Wargin, and R. G. Strate, *Clin. Pharmacol. Ther.*, **21**, 362 (1977).

(3) W. L. Chiou, G. W. Peng, and R. L. Nation, *J. Clin. Pharmacol.*, **18**, 266 (1978).

(4) W. L. Chiou, S. M. Huang, and Y. C. Huang, *Int. J. Clin. Pharmacol.*, **18**, 1 (1980).

(5) W. L. Chiou and F. H. Hsu, *Res. Commun. Chem. Pathol. Pharmacol.*, **10**, 315 (1975).

(6) W. L. Chiou, M. A. F. Gadalla, and G. W. Peng, *J. Pharmacokinet. Biopharm.*, **6**, 135 (1978).

(7) W. L. Chiou, *J. Pharm. Sci.*, **67**, 1776 (1978).

(8) M. Barzegar-Jalali, *Int. J. Pharm.*, **9**, 349 (1981).

(9) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, p. 28.

(10) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, p. 71.

(11) R. V. Churchill, "Operational Mathematics," 2nd ed., McGraw-Hill, New York, N.Y., 1958, p. 157.

(12) C. M. Metzler, G. L. Elfring, and A. J. McEwen, *Biometrics*, **30**, 562 (1974).

(13) C. M. Metzler, G. L. Elfring, and A. J. McEwen, "A Users Manual for NONLIN and Associated Programs," The Upjohn Co., Kalamazoo, Mich., 1974.

(14) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975, Chap. 5.


# Structure Determination of Polysaccharides in *Aloe saponaria* (Hill.) Haw. (Liliaceae)


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**Abstract** □ Neutral polysaccharides that inhibit carrageenin-induced edema in rats were isolated from the nondialysate of the pulp of *Aloe saponaria* by gel filtration. These were shown to be a linear polymer of a 1,4-linked β-D-mannopyranose (mol. wt. 15,000) containing 18% acetyl groups (As mannan 1), and a 1,4-linked α-D-mannopyranose polymer containing a single branch on the principal chain consisting of D-glucose residues linked at C-2 and C-4 (mol. wt. 66,000), with 10% acetyl groups (As mannan 2). As mannan 1 inhibited carrageenin-induced hind paw edema at 50 mg/kg ip in rats; As mannan 2 was not tested for pharmacological activity. A crude preparation of both As mannans was effective when given intraperitoneally, but was ineffective when given orally.



**Keyphrases** □ *Aloe saponaria*—isolation of neutral polysaccharides, As mannan 1, As mannan 2, structural determinations □ As mannans—isolation from *Aloe saponaria*, structural determinations, inhibition of carrageenin-induced edema in rats □ Edema, carrageenin-induced—inhibition in rats by neutral polysaccharides isolated from *Aloe saponica*, As mannan 1, As mannan 2


Pharmacological evidence for an anti-inflammatory effect was provided by the isolation from a nondialysate fraction of *Aloe saponaria*[1] of a glycoprotein with kininase activity (1). Further studies on the nondialysate led to the isolation of neutral polysaccharides that inhibit carrageenin-induced edema in rats. This paper describes the structural studies of the polysaccharides, As mannans 1 and 2, and the pharmacological evaluation of As mannan 1.

[1] This plant is also called spotted aloe or soap aloe.

## EXPERIMENTAL[2]

**Materials**—The following materials were obtained commercially: agarose gel[3], diethylaminoethyl cellulose[4], dialysis membrane[5], bromelain[6], protease[7], anti-inflammatory drug[8], and a series of dextrans[9].

**Methods of Analysis**—The carbohydrate content of the sample was determined colorimetrically by the phenol–sulfuric acid method (2). Elution of material through gel[3] and diethylaminoethyl cellulose[4] filtration was monitored by absorbance of the effluent at 490 nm. Acid hydrolysis was carried out with 1M $H_2SO_4$ at 90°C for 2 h. Excess sulfuric acid was removed by precipitation as barium sulfate. The filtrate was passed through an ion-exchange column[10] to remove the remaining salts, and then the filtrate was evaporated to dryness. Partial hydrolysis was performed with 0.5M $H_2SO_4$ at 70°C for 4 h. Analysis of the carbohydrates by methylation was performed according to the method of Hakomori (3). This procedure was performed twice, to give permethylate showing no hydroxyl absorption in the IR spectrum. Paper partition chromatography[11] of the sugar moiety was performed using butanol–pyridine–water (6:4:3) and aniline hydrogen phthalate detection. Ultracentrifugation[12] was performed on an analytical ultracentrifuge with

[2] The IR spectra were obtained with a KOKEN DS-301 spectrometer. The $^1H$- and $^{13}C$-NMR spectra were recorded with a JEOL PS-100 (100 MHz) and a JEOL FX-100 (25 MHz) spectrometer, respectively. Chemical shifts were recorded in δ units relative to the internal standards 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt or tetramethylsilane. Optical rotations were obtained on a JASCO DIP-4.

[3] Sepharose 6B; Pharmacia Fine Chemicals, Uppsala, Sweden.

[4] DE 32; Whatman Ltd., England.

[5] Visking tube; Union Carbide, Co.

[6] Nakarai Chemical Co., Ltd., Kyoto, Japan.

[7] Type III from papaya; Sigma Chemical Co.

[8] Indomethacin.

[9] Pharmacia Fine Chemicals, Uppsala, Sweden.

[10] Amberlite MR-3; Rohm and Hass Co., Ltd.

[11] Toyo Filter Paper No 50; Toyo Roshi, Co., Tokyo.

[12] Spinco Model E; Beckman Instrument Inc.




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