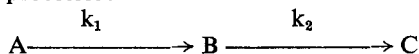


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Mathematics of three-phase *in vitro* absorption models

SIR,—The use of three-phase *in vitro* models and compartmental kinetics to study the drug absorption process would appear to be of great value to pharmacy. Recent reports by Perrin (1966, 1967), who used such a model, contain an error. Perrin pointed out that if the volumes of the compartments within a system differed, they must be taken into account in any kinetic analysis of data obtained from the system. The example used was for the following set of consecutive first order processes:



in which A, B and C represent the concentrations in three compartments with volumes V_A , V_B and V_C . The differential equations describing the kinetics were then given as:

$$V_A dA/dt = -V_A k_1 A \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (1)$$

$$V_B dB/dt = V_A k_1 A - V_B k_2 B \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (2)$$

$$V_C dC/dt = V_B k_2 B \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (3)$$

Using the conditions that $V_A = V_C$, and that at time zero, $A = A_0$, $B = C = 0$, the integrated equations were given as:

$$A = A_0 \exp^{-k_1 t} \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (4)$$

$$B = \frac{V_A k_1 A_0}{V_B(k_2 - V_A k_1/V_B)} (\exp^{-V_A k_1 t} - \exp^{-k_2 t}) \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (5)$$

$$C = A_0 \left[1 - \frac{1}{k_2 - V_A k_1/V_B} (k_2 \exp^{-k_1 t} - k_1 \exp^{-k_2 t} - \frac{V_A k_1}{V_B} \exp^{-k_1 t} + k_1 \exp^{-V_A k_1 t/V_B}) \right] \quad \dots \quad (6)$$

Eqn 4 results from a straightforward integration of eqn 1 and is correct as given. Eqns 5 and 6, however, cannot be obtained by integrating the corresponding differential eqns 2 and 3. The volume terms in the integrated equations are inconsistent with the differential equations. This can be shown by inserting eqn 4 into eqn 2 to give:

$$dB/dt = \frac{V_A}{V_B} k_1 A_0 \exp^{-k_1 t} - k_2 B \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (7)$$

$$\text{or } dB + k_2 B dt = \frac{V_A}{V_B} k_1 A_0 \exp^{-k_1 t} dt \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (8)$$

which is a linear differential equation of the first order. It can, therefore be

integrated through use of an integrating factor (Rainville, 1959). The integrating factor is equal to $\exp^{k_2 \int dt}$ or $\exp^{k_2 t}$. The equation thus becomes:

$$B \exp^{k_2 t} = \frac{V_A k_1 A_0}{V_B} \int \exp^{k_2 t} \exp^{-k_1 t} dt + K \quad \dots \quad (9)$$

in which K is a constant of integration. Using the condition that at time zero, $B = 0$, the equation can be integrated readily and re-arranged to give:

$$B = \frac{V_A k_1 A_0}{V_B (k_2 - k_1)} (\exp^{-k_1 t} - \exp^{-k_2 t}) \quad \dots \quad (10)$$

Consecutive first order reactions involving three species are treated in all standard textbooks of chemical kinetics, including Frost & Pearson (1961). The only difference between reaction kinetics and compartmental kinetics would be that volume terms are included in the differential equations for the latter if the volumes of the compartments differ. Thus, the standard differential equation for reaction kinetics, analogous to eqn 2 would be:

$$dB/dt = k_1 A - k_2 B \quad \dots \quad (11)$$

and the solution is generally given as:

$$B = \frac{k_1 A_0}{k_2 - k_1} (\exp^{-k_1 t} - \exp^{-k_2 t}) \quad \dots \quad (12)$$

Equations 11 and 2 differ only in the appearance of the V_A/V_B term in eqn 2. Thus it might be logical to assume that the solution to eqn 2 could be obtained by inserting the term $V_A k_1/V_B$ wherever k_1 appears in the solution to eqn 11, which is eqn 12. This would indeed yield eqn 5. Thus, the comparison of apparently analogous equations instead of integrating eqn 2 might yield the incorrect eqn 5, instead of the correct eqn 10.

Since eqn 3 can only be integrated using the solution to eqn 2, the use of eqn 5, if it is not the correct solution, would indicate that eqn 6 is also not correct. The correct solution to eqn 3 can be found as follows:

$$dC/dt = \frac{V_B k_2 B}{V_C} = \frac{V_A k_2 k_1 A_0}{V_C (k_2 - k_1)} (\exp^{-k_1 t} - \exp^{-k_2 t}) \quad \dots \quad (13)$$

in which the term B was replaced with eqn 10. This equation can be integrated in a straightforward manner under the conditions that $V_A = V_C$, and that at time zero, $C = 0$ to give:

$$C = A_0 + \frac{k_1 A_0}{(k_2 - k_1)} \exp^{-k_2 t} - \frac{k_2 A_0}{(k_2 - k_1)} \exp^{-k_1 t} \quad \dots \quad (14)$$

which can be re-arranged to give the final equation:

$$C = A_0 \left[1 + \frac{1}{k_1 - k_2} (k_2 \exp^{-k_1 t} - k_1 \exp^{-k_2 t}) \right] \quad \dots \quad (15)$$

There are a number of means whereby the validity of eqns 5 and 10 can be tested. Differentiating them and setting the derivatives equal to zero, will give the time, t_{\max} , at which B reaches a maximum. Thus, for eqn 5:

$$t_{\max} = \frac{1}{k_2 - V_A k_1/V_B} \ln \frac{k_2}{V_A k_1/V_B} = 2.6 \text{ hr} \quad \dots \quad (16)$$

and from eqn 10:

$$t_{\max} = \frac{1}{k_2 - k_1} \ln \frac{k_2}{k_1} = 3.6 \text{ hr} \quad \dots \quad (17)$$

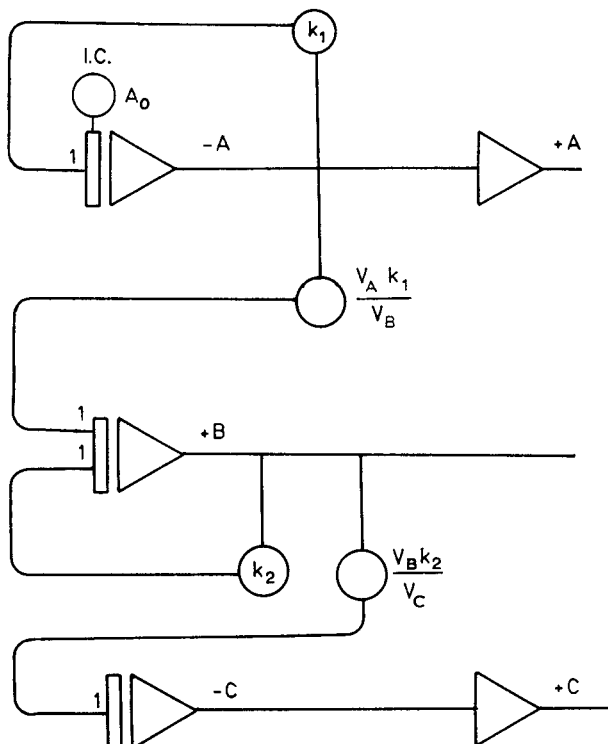


FIG. 1. Analogue computer programme for the solution to eqns 1-3. The time constant was chosen to be 1.00. $A_{\max} = B_{\max} = C_{\max} = 30$ mg/litre.

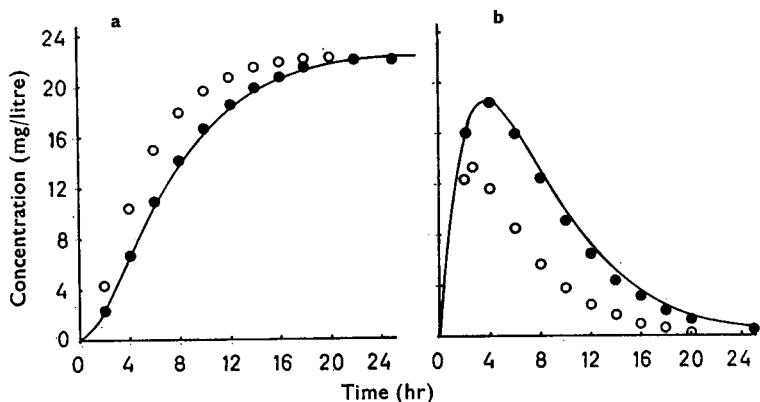


FIG. 2 (a). Plots of C versus time for transfer of salicylic acid from pH 2.0 to pH 7.4 through a layer of 30% decanol in cyclohexane. Constants used were: $k_1 = 0.34 \text{ hr}^{-1}$, $k_2 = 0.22 \text{ hr}^{-1}$, $V_A = 550 \text{ ml}$, $V_B = 300 \text{ ml}$, and $V_C = 550 \text{ ml}$. The line represents the analogue computer solution, ● represents eqn 15 and ○ represents eqn 6. (b) Plots of B versus time for transfer of salicylic acid from pH 2.0 to pH 7.4 through a layer of 30% decanol in cyclohexane. Constants used were: $k_1 = 0.34 \text{ hr}^{-1}$, $k_2 = 0.22 \text{ hr}^{-1}$, $V_A = 550 \text{ ml}$, $V_B = 300 \text{ ml}$. The line represents the analogue computer solution, ● represents eqn 10, and ○ represents eqn 5.

We realize that the reduction in size that results from scaling a submitted figure down to publication size would lead to a loss in accuracy in obtaining t_{\max} from the published figure. However, Fig. 3 from Perrin (1967) would lead us to believe that the maximum in B is reached much closer to 3.6 hr than 2.6 hr (estimated from the Figure is 3.6 hr). Thus, we can be fairly certain that eqn 10, rather than eqn 5 is correct.

Assuming that eqns 1, 2 and 3 are correct, an analog computer programme can be written and a tracing of B versus time can be plotted on an X-Y recorder using the published values for the rate constants and the volumes (Perrin, 1967). Eqns 5 and 10 can also be solved numerically using the same constants and various values of time to give values for B as a function of time. This was done with the analogue computer (Pace Tr-20) programme being shown in Fig. 1. The various values for B versus time are shown in Fig. 2. Since the computer tracing for B versus time represents the integration of eqn 2, any integrated equation which is claimed to be the solution to eqn 2 must yield data matching the computer tracing for B versus time. Fig. 2 shows clearly that eqn 10 is the correct solution to eqn 2, while eqn 5 cannot be correct. The value for t_{\max} from Fig. 2 is found to be 3.6 hr which agrees with the value obtained from eqn 17, as indeed it should. The value of $t_{\max} = 3.6$ hr can be substituted into eqn 10 to find B_{\max} , and $t_{\max} = 2.6$ hr can be substituted into eqn 5 to find B_{\max} . The value of $B_{\max} = 18.4$ mg/litre from eqn 10 is in perfect agreement with the value of 18.4 mg/litre obtained from the computer drawn line in Fig. 2. The value of $B_{\max} = 13.4$ mg/litre, from eqn 5, does not agree at all with the result produced by the computer integration of eqn 2.

The same treatment for C versus time is shown in Fig. 3. Here it is obvious that eqn 15, rather than eqn 6 is the correct solution for eqn 3.

If the model described by eqns 1-3 is to be used for drug absorption studies in three-phase *in vitro* systems, and the integrated expressions are to be used for discussion of the results, we suggest that eqns 10 and 15 be used instead of eqns 5 and 6.

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July 26, 1967

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