Whether or not combinations of other oils and surfactants will exhibit activity with different characteristics has yet to be investigated.

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A Simple Dilution Analog Computer for Simulation of Drug Distribution Processes

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Abstract □ A simple technique is described which provides plots of simulated tandem first-order processes much like the analog computer. The exponential change in concentration of a solution undergoing dilution at constant volume provides the basis for simulation of first-order processes. A UV-absorbing indicator is introduced into the first of a series of connected containers and the solution is rapidly pumped through the system. The continuously changing indicator concentration in any container can be measured and continuously monitored by a spectrophotometer equipped with a flow cell and recorder. Several pharmacokinetic models, as in delayed-release formulation and in molecular modification, were studied with this device and the resulting plots were found to be accurate and reproducible. The validity of dilution analog simulation of first-order processes is shown mathematically. One of the uses of the dilution analog simulator is in teaching and visualizing tandem first-order reactions such as pharmacokinetic models.

Keyphrases □ Analog computer simulation—drug distribution processes □ Model, three compartment—drug distribution □ Kinetic equations—drug distribution model □ Diagrams—models, drug distribution □ UV spectrophotometry—distribution monitoring

The analog computer has been used widely to simulate and analyze drug distribution models (1–3). This report illustrates how first-order dilution techniques with the aid of a spectrophotometer can be used in place of an electronic analog computer to simulate drug transport and distributional systems. In essence, the dilution technique consists of addition of diluent at a constant rate to a well-stirred aqueous solution of a UV-absorbing indicator in which constant volume is maintained by means of an overflow vent. The concentration of indicator decreases exponentially in such a system and additional compartments can be added, the indicator concentrations of which are also governed by exponential laws.

Nonelectronic analog simulation for the purpose of teaching and illustration has been achieved by several ingenious but complicated methods. Krüger-Thiemer (3) used the principle of gas diffusion through evacuated compartments separated by porous membranes. Wendell (4) used a hydrodynamic analog in which the exponential decline of hydrostatic pressure produced a first-order emptying process. Both methods require special equipment and are not easily monitored.

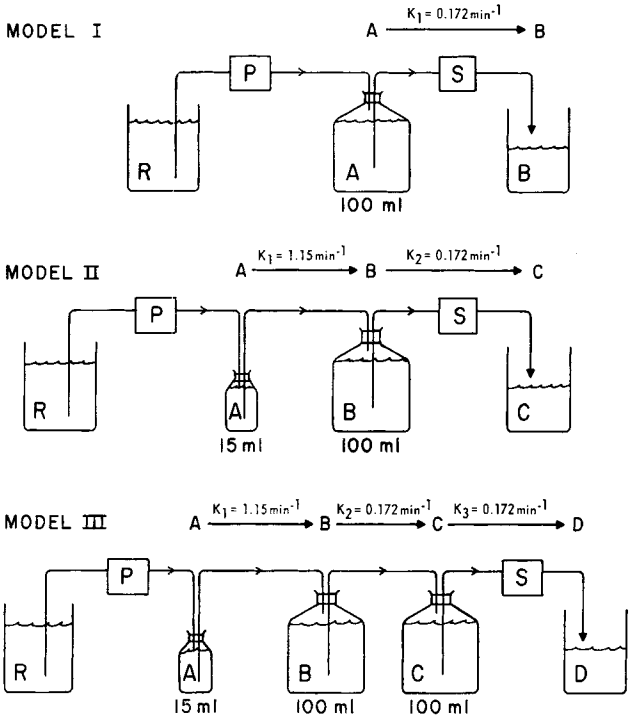

Figure 1—*Models for tandem first-order processes.* P *is a pump which delivers fluid from reservoir* R *to compartment* A *forcing solute solution through the other compartments (flow rate = 17.2 ml. min.*$^{-1}$*).* S *is a spectrophotometer with a flow cell.*

Dilution analog simulation has few disadvantages compared with these systems. The equipment required, including a spectrophotometer, is readily available in most laboratories. While this study deals mainly with systems of tandem first-order processes, the dilution analog technique is not necessarily restricted to such simple systems. Variations in the techniques should permit the simulation of more complex cases such as those involving simultaneously both forward and re-

Table I—Comparison of Equations for Drug (Indicator) in the Various Compartments of the Model Involving Two Consecutive First-Order Processes

$$A \xrightarrow[F/V_A]{k_1} B \xrightarrow[F/V_B]{k_2} C$$

Compartment	*In Vivo*	Dilution-Analog Computer
A	Drug in solution in G.I. tract: $X_A = X_A^\circ e^{-k_1 t}$	Indicator in solution in first constant volume compartment: $X_A = X_A^0\, e^{-(F/V_A)t}$
B	Drug in blood: $X_B = \frac{k_1 X_A^\circ}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t})$	Indicator in solution in second constant volume compartment: $X_B = \frac{(F/V_A)X_A^\circ}{F/V_B - F/V_A}(e^{-(F/V_A)t} - e^{-(F/V_B)t}$
C	Excreted drug: $X_C = X_A^\circ - X_A - X_B$	Cumulative indicator in third compartment: $X_C = X_A^\circ - X_A - X_B$

X_A, X_B, X_C = amount of drug (or indicator) in respective compartment at time t
X_A° = initial amount of drug present in Compartment A at time $t = 0$
k_1, k_2 = first-order rate constants
F = flow rate of fluid
V_A, V_B = fluid volumes of Compartments A and B, respectively

verse rates between compartments as well as parallel pathways.

THEORY

The operation of the dilution-analog computer can be described by a treatment of the case of two consecutive first-order processes:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

where Compartments A, B, and C contain a UV-absorbing indicator in solution with concentrations C_A, C_B, and C_C respectively, and k_1 and k_2 are first-order rate constants for transfer of the indicator from one Compartment to the next. Model II in Fig. 1 shows the basic physical arrangement of the equipment for the simulation of this case. At time zero, Compartment (bottle) A contains all of the indicator in solution while Compartment B is filled to a fixed volume with diluent, and C is empty. The volumes of fluid in compartments A and B are maintained constant since they are tightly stoppered and the air head space is constant and minimal. Solvent is pumped at a constant rate into Compartment A forcing out indicator solution at an equal rate into bottle B. The overflow from B empties into C. Compartments A and B are kept homogeneous by constant stirring with magnetic stirrers. The pumping rate is kept constant throughout the experiment. Either compartment A or B can be monitored; in Model II the spectrophotometer cell is in position to monitor the indicator concentration in Compartment B.

Adjustments of the pumping flow rate and volumes of Compartments A and B can be made to change the rate constants k_1 and k_2. The relationship of these parameters with the rate constants is determined by deriving the equations appropriate to the system as follows.

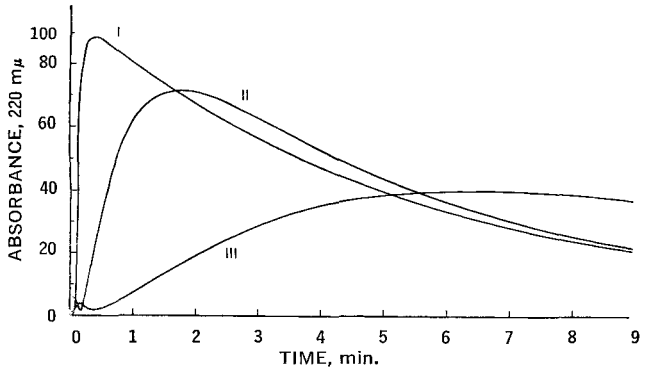

Figure 2—*Recorder tracings obtained from dilution analog computer set up according to Models, I, II, and III in Fig. 1.*

Compartment A—Compartment A contains a fixed volume of indicator solution with indicator concentration C_A which is kept homogeneous by stirring. Dilution fluid (0.116% ammonium hydroxide in aqueous solution in this study) is pumped at a constant flow rate (F ml. min.$^{-1}$) into Compartment A. Since it is tightly stoppered, fluid is forced out of A at the same flow rate F through the outlet tube. In any time interval dt, the flow rate (ml. min.$^{-1}$) multiplied by the time interval dt (min.) gives the volume Fdt (ml.) of fluid pumped into and out of A. The weight (dX_A) of indicator pumped out in the time interval dt is obtained by multiplying the concentration in A (C_A) by the volume pumped out ($C_A Fdt$). Since the fluid pumped into A contains no indicator, the net change in amount of indicator in A is given by

$$dX_A = 0 - C_A\, Fdt \qquad \text{(Eq. 1)}$$

or since $C_A = X_A/V_A$ where X_A = amount of indicator dissolved in the fluid volume (V_A) in Compartment A

$$dX_A = -\frac{X_A}{V_A} Fdt \qquad \text{(Eq. 2)}$$

Integrating:

$$\ln X_A = -\frac{F}{V_A} t + \text{constant} \qquad \text{(Eq. 3)}$$

Evaluation of the constant by equating $t = 0$,

$$\ln \frac{X_A}{X_A^0} = -\frac{F}{V_A} t \qquad \text{(Eq. 4)}$$

or

$$X_A = X_A^0\, e - \left(\frac{F}{V_A}\right) t \qquad \text{(Eq. 5)}$$

where X_A^0 is the initial amount of indicator in compartment A at time $t = 0$.

Compartment B—Using a similar reasoning process as was done for Compartment A, the change in weight of indicator in Compartment B during a time interval dt is

$$dX_B = C_A Fdt - C_B Fdt \qquad \text{(Eq. 6)}$$

Substituting X_A/V_A for C_A and X_B/V_B for C_B,

$$dX_B = X_a \frac{F}{V_A} dt - X_B \frac{F}{V_B} dt \qquad \text{(Eq. 7)}$$

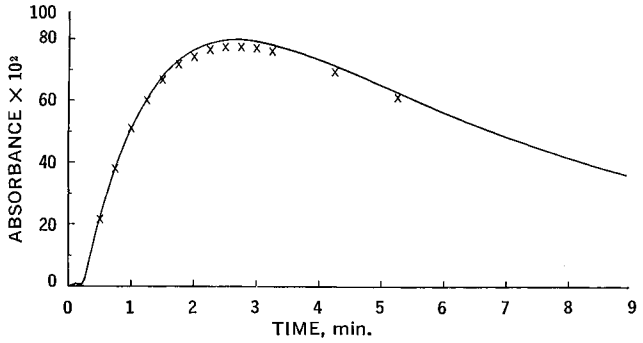

Figure 3—*Simulation of blood level of drug* vs. *time plot applying the* A k_1 → B k_2 → C *model to the dilution analog computer* (k_1 = F/V_A = *0.865 min.*$^{-1}$, k_2 = F/V_B = *0.159 min.*$^{-1}$). *Key:* ——, *experimental recorder tracing;* × *points calculated from Eq. 8.*

Substituting Eq. 5 in Eq. 7 and integrating:

$$X_B = \frac{\left(\frac{F}{V_A}\right)X_A^0}{F/V_B - F/V_A}\left[e^{-\left(\frac{F}{V_A}\right)t} - e^{-\left(\frac{F}{V_B}\right)t}\right] \quad \text{(Eq. 8)}$$

Compartment C—The volume of Compartment C is not constant but continually increases just as does the cumulative urine in a drug distribution process. The complex differential equations are actually unnecessary to consider. Instead, the cumulative weight of indicator excreted into Compartment C can be easily calculated by material balance:

$$X_C = X_A^\circ - X_A - X_B \quad \text{(Eq. 9)}$$

The resultant equations are summarized in Table I and compared with the usual drug distribution equations for a one-compartment model. The equations for amount of indicator (or drug) in Compartments A and B are identical if F/V_A is set equal to k_1 and F/V_B is set equal to k_2. Thus, any set of rate constants can be attained by proper selection of the flow rate F and the volumes V_A and V_B.

EXPERIMENTAL

The models shown in Fig. 1 were constructed from flint glass bottles fitted with two-hole rubber stoppers. The bottles were connected with 0.16 cm. (0.062) in. i.d. glass and rubber tubing. A constant flow rate of fluid was maintained with a peristaltic pump (New Brunswick). A 1-cm. flow cell (Oak Ridge, 0.3 ml. volume) was connected into the system as indicated in the drawing. The absorbance of the liquid in the cell was monitored by a spectrophotometer (Beckman DB) at 220 mμ with a strip chart recorder. The dilution fluid consisted of 0.116% ammonium hydroxide solution and the indicator was 8-nitrotheophylline. The bottles were filled to the desired volumes with the dilution fluid, stoppered, and the pump started. The flow was set at the proper rate and the spectrophotometer was adjusted to a base line of zero. The pump was turned off and with the stirrers adjusted to keep the fluids homogeneous in the compartments, a concentrated solution of 8-nitrotheophylline was quickly injected into Compartment A, allowing about 1 min. to mix with the dilution fluid. The pump and recorder were turned on simultaneously at the beginning of a run. Three milliliters of concentrated 8-nitrotheophylline solution were injected into the first compartment in each case. Each run required about 10 min.

RESULTS AND DISCUSSION

The models in Fig. 1 can be used to illustrate simple drug distribution systems which consist of consecutive first-order processes. The time scale of hours, normal to drug distribution, is reduced to minutes in the models for rapid evaluation. In each case, the bottle which represents the blood compartment is monitored by the spectrophotometer.

Model I in Fig. 1 simulates the case of a drug given i.v. with an excretion half-life of 4.0 hr. The scaled-down rate constant, k_1 = 0.172 min.$^{-1}$, is obtained by adjusting the pumping rate to 17.2 ml. min.$^{-1}$ and the volume of Compartment A to 100 ml. Thus F/V_A = 17.2/100 = 0.172 min.$^{-1}$. The experimental curve obtained is shown in Fig. 2 (Curve I). There is a small lag time due to hold-up volume in the lines.

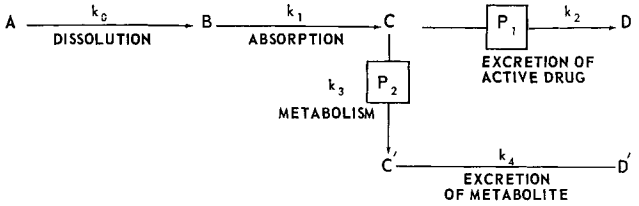

Figure 4—*Complex model for simulation of drug distribution with the dilution-analog computer. Key:* A, B, C, D, C′, D′, *Compartments with volumes* V_A, V_B, etc.; P_1 *and* P_2, *pumps with flow rates* F_1 *and* F_2, *respectively:* k_0, k_1, k_2, k_3, *and* k_4, *rate constants for drug transfer.* k_0 (*if zero-order*) = $(F_1 + F_2)C_A$, k_0 (*if first-order*) = $(F_1 + F_2)/V_A$, $k_1 = (F_1 + F_2)/V_B$, $k_2 = F_1/V_C$, $k_3 = F_2/V_C$, $k_4 = F_2/V_C'$.

Model II simulates the process of absorption and excretion with half-lives of 0.60 and 4.0 hr., respectively. The flow rate of 17.2 ml. min.$^{-1}$ divided by the volume of compartment A, 15 ml., gives the required k_1 of 1.15 min.$^{-1}$. k_2 is numerically the same as k_1 in Model I. The recorder tracing for this system (Curve II in Fig. 2) has the typical blood level curve shape.

Model III simulates the case of a drug derivative which undergoes absorption, hydrolysis to the parent drug, and excretion, with the respective half-lives of 0.60, 4.0, and 4.0 hr. The appropriate rate constants are obtained as before by dividing the flow rate by the bottle volume, *i.e.*, $F/V_A = k_1$, $F/V_B = k_2$, $F/V_C = k_3$. The spectrophotometer is in position to monitor Compartment C, and the absorbance *versus* time curve simulates the concentration of parent drug in the blood. Curve III in Fig. 2 has a prolonged plateau due to the inclusion of the slow hydrolysis step. Of course this model fits other cases such as first-order dissolution in the gut followed by absorption and excretion. The above models show the potential of graphic illustration of various pharmacokinetic models by the dilution-analog computer.

The accuracy of the dilution analog computer was checked by comparison of an experimental curve from Model II with calculated points from the equation in Table I for Compartment B. Rate constants k_1 = 0.865 hr.$^{-1}$ and k_2 = 0.159 hr.$^{-1}$ were taken from an analysis of a clinical study on lincomycin hydrochloride monohydrate (Lincocin) (5). With a fluid flow rate of 13.0 ml. min.$^{-1}$, F/V_A was made numerically equal to k_1 (changing hr.$^{-1}$ to min.$^{-1}$) by adjusting V_A to 15.0 ml. and F/V_B was made equal to k_2 by taking the fluid volume of V_B at 81.6 ml. Since the experimental curve is in absorbance units which is a function of concentration, the calculated values for X_B which are weights must be multiplied by a volume ratio factor, V_A/V_B, to change to a concentration basis while maintaining the equality of Eq. 8.[1]

Figure 3 shows that the fit of calculated points on the experimental curve is very good in spite of the possible experimental errors involved in measuring concentration, volume, and flow rate. The curve approximates very well the actual clinical blood levels.

Simulation with the dilution-analog computer is not limited to these simple systems. Additional consecutive first-order processes can easily be added. An initial zero-order process can be simulated by pumping a solution of indicator at a constant rate into the first fixed volume compartment. Alternate pathways for metabolism and excretion resulting in a branched model are easily visualized. Figure 4 illustrates a more complex model involving an initial zero-order rate and a metabolism step which requires one more pump. Reversible processes require more imagination in design but should be entirely possible. Thus, storage compartments with reversible pathways to the blood compartment could be simulated by a bottle containing an adsorbent or an immiscible solvent for partitioning the indicator with the necessary extra pumps. The rate constants would be functions of the flow rates, compartment volumes, and adsorptive capacities.

[1] To convert X_B and X_A^0 in Eq. 8 to concentration units, both sides are divided by $V_A \cdot V_B$. Algebraic manipulation to keep X_B/V_B or C_B on the left side requires that the right side be multiplied by V_A/V_B.

The authors recognize that the dilution analog computer has limitations with respect to ease of manipulation and applicability to very complex models. It does have the advantages of simplicity, low cost, and availability.

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Mechanism of Hemolysis by Cationic Surface-Active Agents

TAMOTSU KONDO and MICHIKO TOMIZAWA

Abstract □ A study was made of the mechanism of hemolysis by cationic surface-active agents. Phospholipids were found to be released from the cell membrane prior to lysis by the cationic agents under the conditions of physiological pH and ionic strength, indicating an important role of electrostatic interaction between the surface-active cations and the phospholipid anions in hemolysis. The adsorption experiments showed that the binding of surface-active cations to the lipoprotein layer of cell membrane is a prerequisite for the hemolysis to take place. The temperature dependence of adsorption suggested a hydrophobic nature of the binding. It was proposed that the adsorbed surface-active cations make the channels through which the phospholipid molecules are removed out of the cell membrane and that the removal of phospholipids from the lipoprotein layer causes the alteration of protein conformation, thereby allowing the release of hemoglobin into the surrounding medium.

Keyphrases □ Hemolysis mechanism—cationic surfactants □ Cell membrane phospholipids—surfactant effect □ Phospholipid release—cell lysis □ Temperature dependence—membrane-surfactant binding □ TLC—separation, identification

Previous reports (1–3) from this laboratory have revealed that the surface-active cations with alkyl chain length from C_1 to C_{12} are able to cause hemolysis of dog red cells under the conditions of physiological pH and ionic strength, liberating phospholipids in considerable amount from the cell membrane prior to lysis. This has led us to a hypothesis that the surface-active cations strongly interact with the phospholipids in the cell membrane to give rise to hemolysis.

In this paper an attempt was made to examine the validity of the hypothesis in the case of red cells from other animal species than dog and to construct a common mechanism of hemolysis by cationic surface-active agents responsible for all kinds of red cells based on the experimental results obtained.

MATERIALS AND METHODS

Materials—Alkyl pyridinium iodides were synthesized and purified as previously described (4). Alkylamine hydrochlorides and polymethylenediamine dihydrochlorides were prepared by passing dried hydrogen chloride through the benzene solutions of the corresponding amines and diamines. The precipitated salts were recrystallized from ethanol.

Table I—Hemolytic Concentrations of Cationic Surface-Active Agents (m*M*/l.)

Compd.	Species: Dog	Rabbit	Sheep
$C_{12}H_{25}NH_2HCl$	3.41×10^{-1}	2.75×10^{-1}	5.23×10^{-1}
$C_{10}H_{21}NH_2HCl$	2.97	1.88	3.85
$C_8H_{17}NH_2HCl$	3.43×10	2.23×10	4.50×10
$C_6H_{13}NH_2HCl$	4.50×10^2	3.65×10^2	4.58×10^2
$C_{12}H_{25}PyI$	6.20×10^{-1}	4.72×10^{-1}	1.13
$C_{10}H_{21}PyI$	5.40	5.89	7.88
$C_8H_{17}PyI$	3.35×10	3.08×10	3.45×10

The red cell suspension for the experiments was prepared as follows. Citrated blood from normal, healthy animals (dog, rabbit, and sheep) was centrifuged and the cells were washed three times with the phosphate-buffered isotonic saline (pH 7.4) or nonbuffered isotonic saline. The former was used for the hemolysis experiments by alkyl pyridinium iodides, whereas the latter for the ones by alkylamine hydrochlorides and polymethylenediamine dihydrochlorides. The washed, packed cells were then suspended in the same medium as that used in washing to give a 2.5% v/v suspension. The number of cells in unit volume of the suspension was counted on a hemocytometer.

Hemolysis Techniques—The determination of the degree of hemolysis was made in the following way. Various dilutions of cationic surface-active agents in isotonic buffer or saline were made and brought to the required temperature (30°). Two milliliters of each dilution at the same temperature was pipeted into small test tubes, and then an equal volume of the cell suspension was added. The mixtures were allowed to react for 15 min. in the water bath with shaking. At the end of this period, the mixtures were immediately centrifuged to remove the unhemolyzed cells. The degree of hemolysis was evaluated by determining spectrophotometrically the amount of hemoglobin released in the supernatant liquid with a Bausch & Lomb spectrophotometer. The standard for 100% hemolysis was assumed to be given by an optical density value for complete hemolysis. Finally, the hemolysis curve was set up by plotting percent hemolysis against hemolytic agent concentration.

Surface Tension Measurements of Hemolytic Agents Solutions—The surface tension measurements were carried out using a du Nouy tensiometer.

Adsorption of Surface-Active Cations on Red Cells—The amount adsorbed of surface-active cations on dog red cells was estimated