Drug Partitioning III

Kinetics of Drug Transfer in an *In Vitro* Model for Drug Absorption

By JAMES T. DOLUISIO and JOSEPH V. SWINTOSKY

The kinetics of drug transfer from a buffered aqueous phase (compartment A) through a lipid phase (compartment B) to another aqueous buffered phase (compartment C) are reported. These kinetics are obtained from an *in vitro* model system which in important respects mimics drug transfer during the absorptive process. Several kinetic cases were observed—namely, $D_A \rightarrow D_B$, $D_A \rightleftharpoons D_B$, $D_A \rightarrow D_C$, $D_A \rightleftharpoons D_C$, $D_A \rightleftharpoons D_B \rightleftharpoons D_C$, and $D_A \rightleftharpoons D_B \rightarrow D_C$. The $D_A \rightarrow D_C$ and $D_A \rightleftharpoons D_C$ cases are of particular interest since these are often assumed to be applicable to *in vivo* drug absorption. The $D_A \rightarrow D_C$ transfer is actually a subcase of either a $D_A \rightleftharpoons D_B \rightarrow D_C$ or a $D_A \rightarrow D_B \rightarrow D_C$ transfer in which the concentration of D_B is negligible throughout the transfer process. The $D_A \rightleftharpoons D_C$ transfer is actually a subcase of a $D_A \rightleftharpoons D_B \rightleftharpoons D_C$ transfer in which the same condition applies. In these two cases it is evident that the rate of disappearance of D_A equals the rate of appearance of D_C. In other cases where transport into compartment C is occurring there may be an appreciable accumulation of D_B, and only during a steady concentration of D_B are the rates of disappearance of D_A and the appearance of D_C equal. In all experiments the ratio of equilibrium values attained for D_A and D_C agreed with the ratio predicted by the pH partition hypothesis.

THIS PAPER reports the kinetics of drug transfer from an aqueous phase (compartment A) to a lipid phase (compartment B) to another aqueous phase (compartment C). These kinetics are of interest because they are obtained from an *in vitro* model system which in important respects mimics drug transfer during the absorption process.

During the past several decades much attention has been given to clarifying the relationships between chemical structure and the physical-chemical properties of compounds, such as solubilities in various solvents, partition coefficients, and melting points. More recently, phenomena such as drug absorption, excretion, and other biologic phenomena have been rendered more understandable as a result of correlations of these phenomena with the physical-chemical properties of the compounds and the tissues to which they have access.

Experimental techniques for studying factors which influence rates of drug transfer and equilibrium distribution, for example, in the process of absorption, involve both *in vivo* and *in vitro* methods. Usually the *in vivo* methods for studying absorption in man or animals involve measurements of drug disappearance from the gastrointestinal tract. Sometimes the rate of entry of the drug into the blood is measured by collecting blood directly from the mesentery circulation. In some instances blood and urine data are used to make estimates of the kinetics of the drug transfer processes. In no instance is it possible to measure directly concentration of drug in the absorbing gut membranes without sacrificing the animals.

In vitro techniques for studying factors influencing the rate of drug transfer processes have usually employed the isolated gut of a small animal such as a rat or hamster. This valuable technique suffers from the fact that the isolated gut, without intact blood supply, deteriorates quite rapidly, losing its integrity within several hours. Also, intestinal segments show some variation in permeability from one animal to another, and drug transfer rates are exceedingly slow. The *in vivo* and *in vitro* methods noted above are obviously important, yet for certain type studies they represent rather complicated ways of obtaining information relative to the time course of drug transfer as it may occur during, for example, passive absorption.

For many drugs absorption appears to occur by diffusion processes without the mediation of enzymes or a requirement for energy. Since a preponderance of data indicates that drug absorption consists of transfer from an aqueous phase, through a membrane which resembles a lipid phase, into the aqueous circulating fluids of the body, the authors have attempted to utilize a somewhat equivalent *in vitro* model, free of living tissue or solid membranes, for kinetic studies of drug transfer. The previously reported (1) inverted Y tube with aqueous phases in each of the two arms, but overlaid with a connecting layer of an immiscible lipid-like liquid, in

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many respects mimics the *in vivo* situation. By introducing drug in one aqueous phase, and slowly rocking the assembly under reproducible conditions, drug transfer occurs in a reproducible manner. The time course of drug transfer and equilibrium distribution can be determined by measuring drug concentrations in each of the three phases, and these studies on a single system can be conducted for hours or days as necessary. An *in vitro* model appears to offer as some of its important advantages the opportunity to study by simple methods the effect of (*a*) varying the type and number of compartments, (*b*) additives in any phase, (*c*) pH, (*d*) drug structure modifications, and (*e*) viscosity on the drug transfer process.

In this paper the authors report the type of kinetics observed for several well-known drugs and show how these kinetics obey the predictions of equations which may be written for drug transfer between the three given compartments.

EXPERIMENTAL

Apparatus and Reagents.—All chemicals were reagent grade unless specified otherwise. Salicylic acid, antipyrine N.F., chlorpromazine ampul grade, barbital N.F., potassium chloride, monobasic sodium phosphate, boric acid, sodium hydroxide, hydrochloric acid, cyclohexane, *n*-octyl alcohol, Leeds and Northrup model 7401 pH meter, and a Beckman model DB spectrophotometer were employed.

The glass tubes, rocking apparatus, and aqueous buffers used in these studies were described in a previous communication (1).

Procedure.—Aqueous buffered solutions of drug (15.0 ml.) were placed in one arm of the tube, 15.0 ml. of a pH 7.4 phosphate buffer solution was placed in the remaining arm, and 85.0 ml. of cyclohexane or *n*-octyl alcohol added. The system was gently rocked and the drug concentrations of the aqueous phases determined at various time intervals. The concentrations of the lipid phase were calculated by difference.

Salicylic acid, antipyrine, and chlorpromazine, were determined spectrophotometrically. Barbital solutions were adjusted to pH 9.4 with a borate buffer, then determined spectrophotometrically.

RESULTS AND DISCUSSION

In general, the rate constants denoting the transfer of drug from A to B, B to A, B to C, and C to B are k_1, k_2, k_3, and k_4, respectively. That is,

$$D_A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D_B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} D_C \qquad \text{(Eq. 1)}$$

where D_A, D_B, and D_C represent the molar concentrations of single drug in the three distinct liquid phase compartments, A, B, and C.

A number of special cases of Eq. 1 are possible, depending upon the values of the rate constants. The following six cases were observed in the *in vitro* model:

Case *1:* $D_A \rightarrow D_C$

Case *2:* $D_A \rightleftharpoons D_C$

Case *3:* $D_A \rightarrow D_B$

Case *4:* $D_A \rightleftharpoons D_B$

Case *5:* $D_A \rightleftharpoons D_B \rightleftharpoons D_C$

Case *6:* $D_A \rightleftharpoons D_B \rightarrow D_C$

The following two cases are theoretically possible but have not been observed as yet in the *in vitro* model:

Case *7:* $D_A \rightarrow D_B \rightarrow D_C$

Case *8:* $D_A \rightarrow D_B \rightleftharpoons D_C$

Case *1:* $D_A \rightarrow D_C$

Figures 1 and 2 illustrate the transfer characteristics of this case. Note that transfer to compartment C is complete, the sum of D_A and D_C accounts for total drug in the system, and the disappearance of D_A is first order. Actually this example is a special case of a $D_A \rightleftharpoons D_B \rightarrow D_C$ process where D_B does not accumulate. Since D_B does remain negligible during the transfer, a steady concentration of D_B can be assumed. That is,

$$\frac{dD_B}{dt} = k_1 D_A - k_2 D_B - k_3 D_B = 0 \qquad \text{(Eq. 2)}$$

Therefore

$$D_B = \frac{k_1}{k_2 + k_3} D_A \qquad \text{(Eq. 3)}$$

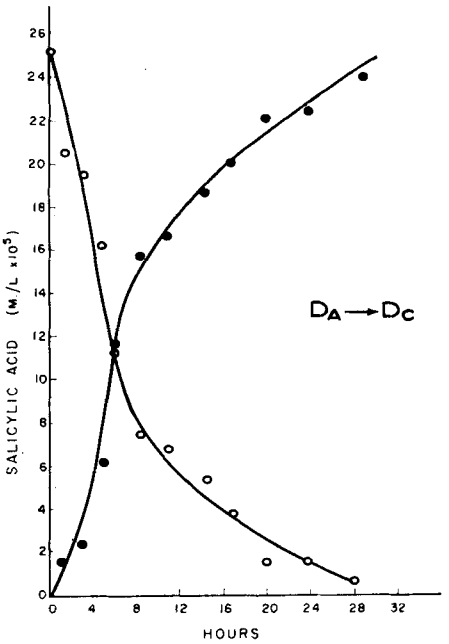

Fig. 1.—The transfer of salicylic acid from an aqueous pH 2.0 phase through a cyclohexane barrier to an aqueous pH 7.4 phase. The concentrations of salicylic acid in cyclohexane were low and could not be accurately determined (*e.g.*, less than 0.4×10^{-6} *M*/L.). Key: O, pH 2.0, phase *A;* ●, pH 7.4, phase *C*.

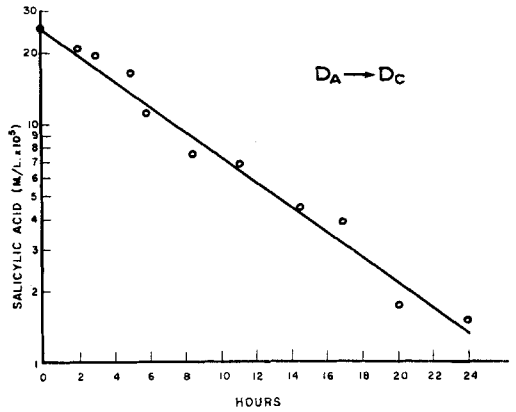

Fig. 2.—A plot of data in Fig. 1, according to Eq. 6, showing the apparent first-order disappearance of salicylic acid from compartment A. Since the concentration of D_B was negligible (thus constant) throughout the experiment, the slope of the line does not vary as it does in Fig. 10.

and the rate of appearance of D_C is related to D_A by

$$\frac{dD_C}{dt} = -\frac{dD_A}{dt} = \beta D_A \qquad \text{(Eq. 4)}$$

where

$$\beta = \frac{k_3 k_1}{k_2 + k_3} \qquad \text{(Eq. 5)}$$

Integration of Eq. 4 yields

$$\ln D_A = -\beta t + \ln D_A^0 \qquad \text{(Eq. 6)}$$

Thus, in this case, the disappearance of D_A is first order, but the apparent rate constant, β, is a function of k_1, k_2, and k_3. If it can be assumed that $k_2 \ll k_3$ or $k_2 = 0$, then β equals k_1. This assumption is not valid for the data in Fig. 1 since it was found that if the drug is initially placed in B, appreciable buildup in A occurred in the early stages of the experiments.

Case *2:* $D_A \rightleftharpoons D_C$

Figures 3 and 4 illustrate the type of transfer characteristic of this case. Note in Fig. 3 that at equilibrium both D_A and D_C are present and that the sum of D_A and D_C accounts for all the drug present in the system. In Fig. 4 note that a plot of log $(D_A - D_A^\infty)$ *versus* time yields a straight line. This example is a special case of a $D_A \rightleftarrows D_B \rightleftharpoons D_C$ transfer where D_B does not accumulate. Thus, like Case *1*, a steady concentration of D_B can be assumed. That is,

$$\frac{dD_B}{dt} = k_1 D_A + k_4 D_C - k_2 D_B - k_3 D_B \qquad \text{(Eq. 7)}$$

Therefore

$$D_B = \frac{k_1 D_A + k_4 D_C}{k_2 + k_3} \qquad \text{(Eq. 8)}$$

and the rate of disappearance of D_A and the appearance of D_C are equal. Thus

$$-\frac{dD_A}{dt} = \frac{dD_C}{dt} = k_1 D_A - k_2 D_B = k_1 D_A - k_2 \frac{k_1 D_A + k_4 D_C}{k_2 + k_3} \qquad \text{(Eq. 9)}$$

Since, in this case D_B is negligible, the approximation that

$$D_A^0 - D_A = D_C \qquad \text{(Eq. 10)}$$

where D_A^0 represents the initial concentration of D_A, is valid. Substituting Eq. 10 into Eq. 9, it can be shown that

$$-\frac{dD_A}{dt} = \alpha D_A - \beta D_A^0 \qquad \text{(Eq. 11)}$$

where

$$\alpha = \frac{k_1 k_3 + k_2 k_4}{k_2 + k_3} \qquad \text{(Eq. 12)}$$

and

$$\beta = \frac{k_2 k_4}{k_2 + k_3} \qquad \text{(Eq. 13)}$$

Integrating Eq. 11 and rearranging yields

$$\ln (\alpha D_A - \beta D_A^0) = -\alpha t + \alpha Z \qquad \text{(Eq. 14)}$$

where Z is a constant of integration.

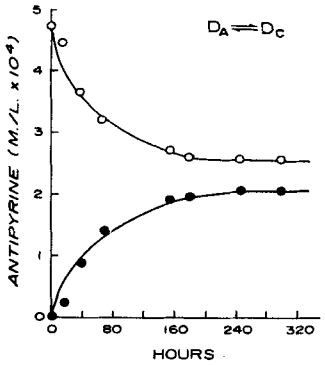

Fig. 3.—The transfer of antipyrine from an aqueous pH 2.0 phase through a cyclohexane barrier to an aqueous pH 7.4 phase. The concentrations of antipyrine in cyclohexane were low and could not be determined accurately. At equilibrium, antipyrine appeared to be present in only phases A and C. Key: O, pH 2.0, phase A; ●, pH 7.4, phase C.

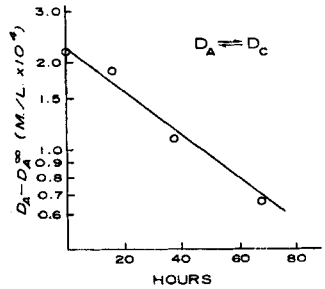

Fig. 4.—A plot of data in Fig. 3, according to Eq. 18. Since the concentration of D_B was negligible (thus constant) throughout the experiment, the slope of the line does not vary as it does in Fig. 8.

At equilibrium Eq. 11 equals 0, thus

$$\alpha D_A^{\infty} = \beta D_A^0 \qquad \text{(Eq. 15)}$$

where D_A^{∞} denotes the concentration of D_A at equilibrium. Substituting Eq. 15 into Eq. 14 and rearranging yields

$$\ln [\alpha (D_A - D_A^{\infty})] = -\alpha t - \alpha Z \qquad \text{(Eq. 16)}$$

From this equation it can be seen that when $t = 0$

$$\alpha Z = \ln [\alpha(D_A^0 - D_A^{\infty})] \qquad \text{(Eq. 17)}$$

Substituting Eq. 17 into Eq. 16 and rearranging yields

$$\ln \left(\frac{D_A - D_A^{\infty}}{D_A^0 - D_A^{\infty}}\right) = -\alpha t \qquad \text{(Eq. 18)}$$

Thus, as shown in Fig. 4, a plot of $\log (D_A - D_A^{\infty})$ *versus* t yields a straight line with a slope of $-\alpha/2.303$ and a Y intercept of $\log (D_A^0 - D_A^{\infty})$.

Case *3:* $D_A \rightarrow D_B$

The transfer in this case is simple first order and obeys the equation:

$$\ln D_A = -k_1 t + \ln D_A^0 \qquad \text{(Eq. 19)}$$

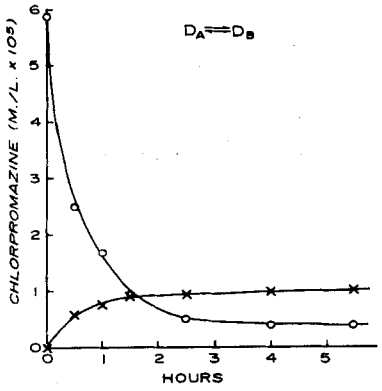

Fig. 5.—The transfer of chlorpromazine from an aqueous pH 2.0 phase to cyclohexane. No transfer to the aqueous pH 7.4 phase occurred and a state of equilibrium developed between chlorpromazine in phases *A* and *B*. Key: O, pH 2.0, phase *A*; ×, cyclohexane phase *B*.

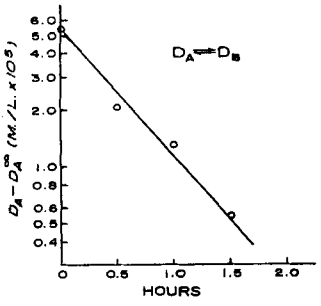

Fig. 6.—A plot of data in Fig. 5, according to Eq. 20, showing a reversible transfer of drug between compartments *A* and *B*. No drug transferred into compartment *C* under the conditions of this experiment.

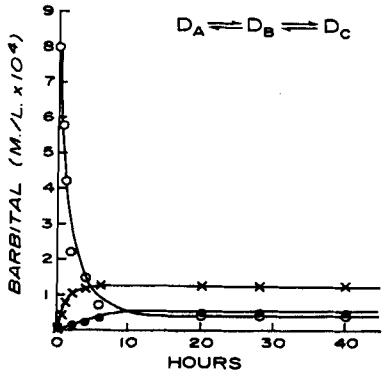

Fig. 7.—The transfer of barbital from an aqueous pH 2.0 phase to an *n*-octyl alcohol phase to an aqueous pH 7.4 phase. A state of equilibrium developed between barbital in phases *A*, *B*, and *C*. Key: O, pH 2.0, phase *A*; ●, pH 7.4, phase *C*; ×, *n*-octyl alcohol, phase *B*.

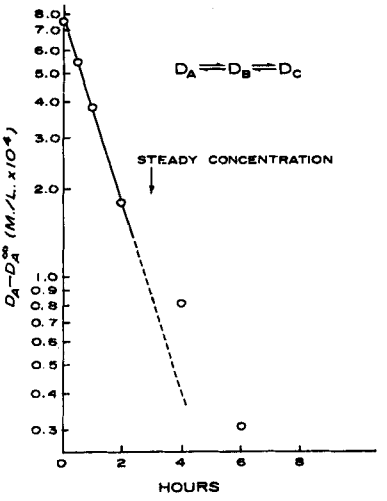

Fig. 8.—Semilog plot of data in Fig. 7. The rate of barbital disappearance from phase *A* changed after a steady concentration of barbital in *n*-octyl alcohol was attained.

Case *4:* $D_A \rightleftharpoons D_B$ (See Figs. 5 and 6.)

The transfer in this case is simple reversible first order and obeys the equation:

$$\ln \left(\frac{D_A - D_A^{\infty}}{D_A^0 - D_A^{\infty}}\right) = -(k_1 + k_2')\, t \qquad \text{(Eq. 20)}$$

where $k_2' = k_2/5.67$. The factor of 5.67 is introduced due to the difference in volume between the aqueous and lipoidal phases. That is,

$$D_A^0 = D_A + 5.67\, D_B \qquad \text{(Eq. 21)}$$

Case *5:* $D_A \rightleftharpoons D_B \rightleftharpoons D_C$ (See Figs. 7 and 8.)

In this case D_A, D_B, and D_C are present at equilibrium.

Case *6:* $D_A \rightleftharpoons D_B \rightarrow D_C$ (See Figs. 9 and 10.)

In this case only D_C is present at equilibrium and D_B accumulates during the experiment. Additional information is required to differentiate this case

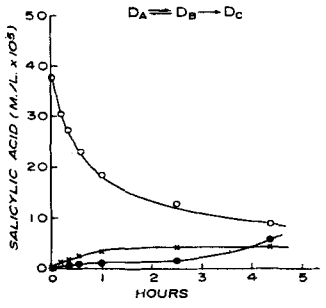

Fig. 9.—The transfer of salicylic acid from an aqueous pH 2.0 phase to an *n*-octyl alcohol phase to an aqueous pH 7.4 phase. At equilibrium, all the drug was present in the pH 7.4 phase. Key: O, pH 2.0, phase *A*; ●, pH 7.0, phase *C*; ×, *n*-octyl alcohol, phase *B*.

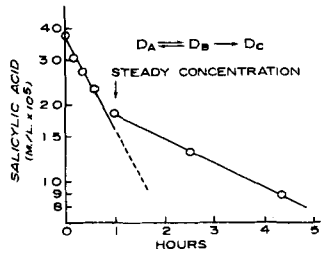

Fig. 10.—A semilog plot of data in Fig. 9. The rate of disappearance from phase *A* changed after a steady concentration of drug in phase *B* was attained.

from Case *7*. The easiest method to determine which case is illustrated is to vary the initial conditions so that the drug is in compartment *B* and determine whether drug transfers to compartment *A*.

In Cases *5*, *6*, *7*, and *8* steady concentration of D_B may or may not exist. It should be noted that only during a steady concentration is the disappearance of D_A equal to the appearance of D_C. Figures 8 and 10 illustrate that the rate of disappearance of D_A changes after a steady concentration of D_B is attained. These kinetics can be solved using an analog computer. Another method is to replace one of the aqueous phases with glass beads and independently study the kinetics of transfer in the two phase systems, for example, $D_A \rightarrow D_B$ systems and $D_B \rightarrow D_C$ systems.

Results of study with this *in vitro* model point to some possible unexpected problems in the experimental methods employed in obtaining *in vivo* data. For example, in *in vivo* studies, the disappearance of drug from the gastrointestinal tract can account accurately for the portion of drug absorbed, but such a measurement does not reveal how much of the drug has entered into the general circulation or how much has been stored in the absorbing membranes. For example, work with the *in vitro* model suggests that for low dose lipophilic drugs, the rate of drug disappearance from the gastrointestinal tract perhaps may not coincide with the rate of drug appearance in the blood.

In all the experimental results, the equilibrium values attained for D_A and D_C agreed closely with the ratio predicted by the pH partition hypothesis (2). Also the results indicate that k_1 and k_4 are functions of pH and pKa. Certain inferences can be drawn regarding the type kinetics that will result when pH, pKa, solubilities, and partition coefficients are known. For example, when drug solubilities in the lipid phase are very poor relative to aqueous solubility in compartment *A*, transfer will be extremely slow from *A* to *B*. Also, when drug in *C* is virtually completely ionized and drug in *A* is only partially ionized, Case *1* or Case *6* kinetics are possible. For this reason, absorption of weak acids from the stomach may mimic Case *1* or *6* kinetics. If a drug is only partially ionized in both *A* and *C*, Case *2* or Case *5* kinetics are probable. For this reason, absorption of weak bases from the intestines might, therefore, follow Case *2* or Case *5* kinetics.

The *in vitro* drug transfer profiles illustrated bear some resemblance to absorption and excretion profiles obtained from the *in vivo* studies. The profile for disappearance of drug from compartment *A* resembles what one observes in studies of drug disappearance from the gastrointestinal tract. The profiles for drug accumulation in compartment *C*, when $k_4 = 0$, resemble what one observes in plots of cumulative urinary excretion data. The profiles for drug concentration in compartment *B* are reflections of steady concentration conditions that may exist *in vivo* in body membranes involved in drug transport. By introducing one or more additional appropriate compartments in an *in vitro* model of this type, it is apparent that one could also mimic blood concentration or other tissue concentration profiles.

The rates of drug transfer in the *in vitro* model often may be rationalized in terms of the physical properties of the compounds and of the aqueous and nonaqueous compartments. For example, it has been shown that the transfer of salicylic acid is much faster than that of barbital when the drugs are partitioning from an aqueous pH 2.0 compartment into cyclohexane (1). Under the conditions of these previous studies, salicylic acid has a much higher o/w pH 2.0 partition coefficient than barbital. However, when *n*-octyl alcohol, a compound capable of hydrogen-bonding with weak acids, replaces cyclohexane, the partition coefficients are somewhat similar. Thus, it is not surprising that the initial rates of drug disappearance from compartment *A* for barbital and for salicylic acid are approximately equal when the lipoidal barrier consists of *n*-octyl alcohol. (See Figs. 8 and 10.)

Thus, on the basis of physical-chemical interrelationships between the drug and liquid phases one can anticipate some of the kinetic effects. However, whether a given drug under the conditions of the study will establish steady concentration of D_B rapidly or at all, whether equilibrium distribution conditions will be attained rapidly or slowly relative to a certain drug, or whether the kinetics will be of one case or another, is difficult if not impossible to anticipate on theoretical grounds because of the number of variables interacting and the occurrence of several simultaneous processes which are occurring at changing rates. Thus, it becomes desirable to have available a moderately simple *in vitro* model simulating some of the events of the *in vivo* system from which appropriate data can be obtained quickly and easily. The drug transfer profiles are then

subjected to the usual computational methods which permit estimation of rate constants.

The authors hope to show more fully in future studies how knowledge acquired from *in vitro* transfer systems can be applied to better understanding of the absorption, distribution, and excretion profiles of drugs studied *in vivo*. This should be possible even though *in vivo* profiles are complicated by the interposition of metabolizing machinery of the cells, extra drug compartments in the body, and excretion apparatus.

Also of much interest is the use of the *in vitro* model to evaluate the effects of placing additives in the various liquid phases or of altering the physical properties of a drug through functional group modifications.

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Rheology of Concentrated Dicalcium Phosphate Suspensions

By JOHN E. BUJAKE, JR.

The flow properties of concentrated (30–42 per cent by volume) dicalcium phosphate dihydrate (DCP) paste suspensions and model systems have been determined over a shear rate range of 0.045 to 6200 sec.$^{-1}$ using concentric cylinder, cone-plate, and capillary extrusion viscometers at 25°. Apparent shear rates were corrected for non-Newtonian flow which gave optimum agreement of the data from the three instruments. Shear stress–shear rate data fitted the empirical power-law equation, $\tau = K\dot{\gamma}^n$, over most regions of the above shear rate range. Flow indices (n) of 0.23–0.55 indicated appreciable shear thinning and suggested significant particle–particle interaction in these suspensions which are similar to consumer toothpastes. The greatest structural effects and non-Newtonian behavior were observed at low shear rates. Flow characteristics of the paste suspensions were comparable to those of a simple DCP–water model system. The effect of electrolytes on the viscosity of the DCP–water system was correlated with sedimentation volume data. The existence of a DCP network structure and reversible time-dependent bonds is postulated.

THE RHEOLOGICAL properties of concentrated paste systems are interesting from the viewpoint of both fundamental and applied research. Work reported in the literature on the quantitative characterization of the flow of highly concentrated suspensions has been somewhat limited. The Goodeve and Williamson equations have been applied with some success to suspensions at limiting high shear rates (1–4). Metzner has examined the flow of concentrated shear thickening suspensions and summarized some of the earlier work (5). Concentrated quartz powder suspensions have been studied by a number of workers (6). A review of some basic concepts of suspension formulation has also been presented (7).

It seemed that additional quantitative data on the flow properties of concentrated suspensions over a wide range of shear conditions would be of use in characterizing, controlling, and predicting the behavior of suspensions under processing and use conditions. Such information would be particularly useful in gaining an understanding of flow in toothpaste and other similar systems.

Rheological investigations are basically concerned with the determination of the relationship between shear stress and shear rate. For Newtonian materials with their constant viscosity, this is relatively simple. However, concentrated suspensions have non-Newtonian and often time-dependent flow characteristics which necessitate a study over a wide range of shear rates under well-defined conditions. Low shear rates are particularly useful in structural studies. Shear stresses and shear rates must be calculated at an identical point in the system, *e.g.*, at the wall, and shear rates must be corrected for non-Newtonian behavior to obtain actual rates of shear.

In this work the non-Newtonian flow characteristics of some concentrated dicalcium phosphate dihydrate (DCP) paste suspensions and model systems have been examined using concentric cylinder, cone-plate, and capillary ex-

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