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Research Articles

Simultaneous Determination of Dissolution and Partitioning Rates *In Vitro*

By P. J. NIEBERGALL, M. Y. PATIL, and E. T. SUGITA

An *in vitro* method is presented for the simultaneous determination of the dissolution and partitioning rates of drugs. The kinetics of the system is described, and a number of methods are given for evaluating the rate constants. The effects of stirring rate and temperature upon the rate constants were investigated and found to be in agreement with what would be expected for diffusion-controlled processes.

THE DISSOLUTION rate of a drug may have a marked effect upon the absorption of the drug from a solid dosage form. This has led to an increasing interest in developing *in vitro* dissolution rate tests that can be correlated with *in vivo* absorption rate studies for possible use in quality control or for use in setting official standards. In addition, dissolution rates have been used to evaluate formulation variables under the assumption that all other things being equal, the drug formulation that dissolves most rapidly would have the greatest chance of clinical success.

The over-all absorption process for solid drugs, however, consists of the dissolution step followed by drug partitioning into an essentially lipid barrier. The three phase "rocking apparatus," developed by Doluisio and Swintosky (1), appears to have great promise in studying the effects of additives or of molecular modification upon the partitioning rates of drugs. This apparatus, however, cannot be used to investigate the dissolution process, and therefore is useful only for drugs in solution. Thus, the over-all process of absorption

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is generally studied *in vitro* as two separate processes, each of which might be rate limiting.

It is conceivable that certain factors might increase the dissolution rate of a drug while simultaneously decreasing its partitioning rate, and possibly reverse the importance of each process in the absorption of the drug. For example, *in vitro* dissolution rate studies have shown that surfactants are capable of increasing the dissolution rate of drugs (2, 3). It has also been shown, however, that surfactants can decrease the *in vivo* absorption of a drug such as salicylic acid (4), the absorption of which would be expected to be rate controlled by the dissolution process. The authors decided, therefore, to investigate the possibility of simultaneously determining the *in vitro* drug dissolution and partitioning rates in a single system.

EXPERIMENTAL

Reagents—Potassium chloride, hydrochloric acid, sodium hydroxide, and salicylic acid were of analytical reagent grade obtained from the J. T. Baker Co. Standard grade *n*-octyl alcohol was obtained from Matheson Coleman and Bell. This grade octanol did not interfere with the spectrophotometric determination of salicylic acid.

Apparatus—The apparatus chosen for the study consisted of a 500-ml. round bottom, three-necked standard taper flask fitted with a mercury seal stirrer in the center opening. A 1-in. three-blade propeller was attached to the bottom of the stirring shaft which was inserted into the chuck of a variable speed stirrer.[1] The bottom edge of a second 1-in. three-blade propeller was placed on the stirring shaft exactly 3 cm. above the upper edge of the lower propeller. When 250 ml. of the aqueous phase was added to the flask, the upper edge of the bottom propeller was 2 cm. below the liquid surface. A large diameter glass tube was inserted into one of the remaining standard taper openings, and the closure sealed with aluminum foil. Octyl alcohol (250 ml.) was added through the remaining opening which was closed with a standard taper glass stopper. The lower edge of the upper propeller was 2 cm. above the octanol–water interface.

Procedure—Hard, nondisintegrating tablets of salicylic acid were prepared on a Carver model B laboratory press, without the aid of a lubricating agent. Four tablets were weighed and used in each study. In no instance was the weight loss permitted to extend beyond 10% (2.5% per tablet), thus enabling the assumption of constant surface area.

The apparatus described above was placed in a water bath until both liquid phases had reached constant temperature. The stirrer was started and adjusted to the desired speed, which was measured with a tachometer. The four tablets were introduced into the pH 2.00 buffer solution (hydrochloric acid–potassium chloride, Clark-Lubs, U.S.P. XVII) through the glass tube. One-

[1] Model 102, Talboys Instrument Co., Emerson, N. J.

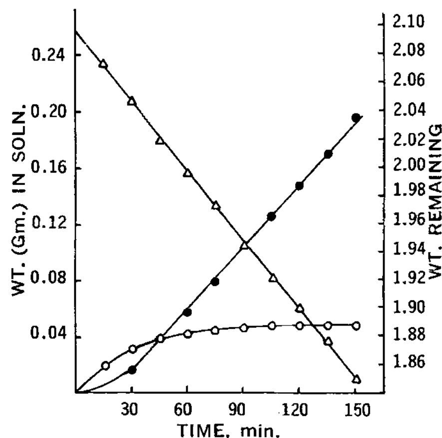


Fig. 1—Dissolution and partitioning rates of salicylic acid at 100 r.p.m. and 30°. Key: △, weight remaining in tablets; ○, weight in pH 2.00 buffer; ●, weight in octanol.

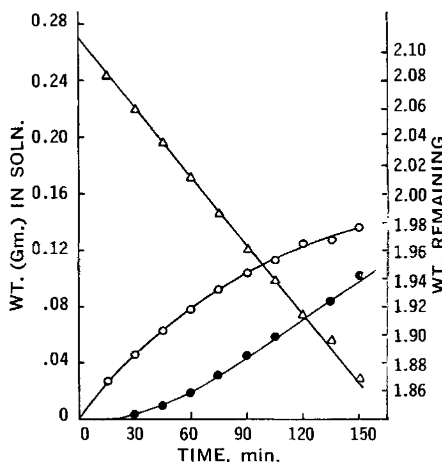
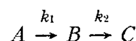


Fig. 2—Dissolution of salicylic acid into 0.1% w/v solution of polysorbate 80 at pH 2.00 and partitioning into octanol. Stirring rate was 100 r.p.m. at 30°. Key: △, weight remaining in tablets; ○, weight in polysorbate 80 solutions; ●, weight in octanol.

milliliter samples of both phases were obtained at 15-min. intervals and assayed at 298 mμ using a Beckman model DU spectrophotometer. The samples were diluted when necessary with either the pH 2.00 buffer or octanol. A total of 10 samples was taken for each run.

RESULTS

Kinetic Analysis of the Data—Typical results obtained are shown in Figs. 1 and 2. The simplest mechanism to be expected in a system such as we have described might be:



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

in which A represents the drug in the tablets, B represents the drug in the aqueous phase, and C represents the drug in the octanol. In this study,

the amount of drug in each compartment is given as weight, rather than concentration, to aid in the evaluation of the constants. This causes no difficulty due to the nature of the dissolution process, and because the volumes in compartments B and C are kept constant. The following equations would describe the kinetics of the proposed mechanism:

$$-\frac{dA}{dt} = k_1 (W_s - B) \quad \text{(Eq. 1)}$$

$$\frac{dB}{dt} = k_1 (W_s - B) - k_2 B \quad \text{(Eq. 2)}$$

$$\frac{dC}{dt} = k_2 B \quad \text{(Eq. 3)}$$

in which W_s is the weight of drug needed to saturate the aqueous phase, and the other terms have the meanings previously given. The solubility of the salicylic acid in the pH 2.00 buffer and in octanol at various temperatures is given in Table I. In the early stage of the dissolution process, $W_s \gg B$ and:

$$-\frac{dA}{dt} = k_1 W_s \quad \text{(Eq. 4)}$$

Thus, a plot of weight remaining *versus* time should give a straight line, as shown in Figs. 1 and 2.

The weight of drug in the aqueous phase as a function of time can be obtained from the following:

$$\frac{dB}{dt} = k_1 W_s - k_1 B - k_2 B = K - K' B \quad \text{(Eq. 5)}$$

in which $K = k_1 W_s$ and $K' = k_1 + k_2$. The use of an integrating factor, and the condition that at time zero, $B = 0$, gives:

$$B = \frac{K}{K'} (1 - \exp.^{-K't}) \quad \text{(Eq. 6)}$$

The method of Guggenheim (5) can be used to obtain K'. Plots for typical data utilizing this method are shown in Figs. 3 and 4. Once the value of K' is obtained by least squares evaluation of the lines, B can be plotted according to Eq. 6 to yield a straight line with a slope equal to K/K'. A knowledge of W_s then enables k_1 and k_2 to be evaluated. Representative plots of Eq. 6 are shown in Figs. 5 and 6.

Steady-State Treatment—The data shown in Fig. 1 indicate that a steady state may be obtained rather quickly in the aqueous phase. If this assumption can be made:

$$\frac{dB}{dt} = 0 = k_1 (W_s - B) - k_2 B \quad \text{(Eq. 7)}$$

TABLE I—SOLUBILITY OF SALICYLIC ACID IN pH 2.00 BUFFER AND IN OCTANOL AT VARIOUS TEMPERATURES

Temp., °C.	Phase	Solubility, Gm./250 ml.
30	pH 2.00 buffer	0.56
30	Octanol	29.44
32.5	pH 2.00 buffer	0.68
32.5	Octanol	31.88
35	pH 2.00 buffer	0.75
35	Octanol	34.32

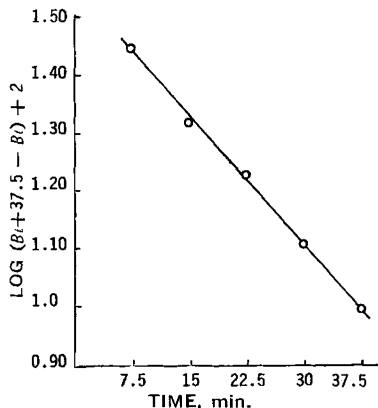


Fig. 3—Guggenheim plot for the appearance of salicylic acid in the pH 2.00 buffer. Stirring rate was 100 r.p.m. at 30°.

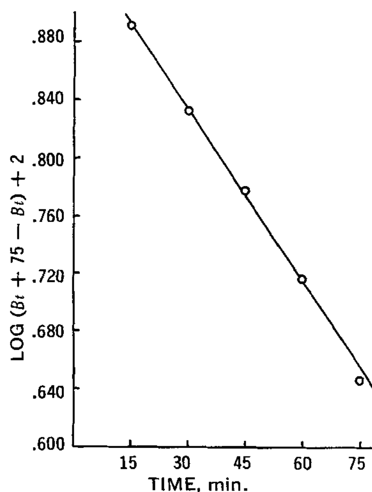


Fig. 4—Guggenheim plot for the appearance of salicylic acid in the 0.1% w/v polysorbate 80 solution at pH 2.00. Stirring rate was 100 r.p.m. at 30°.

and therefore:

$$k_2 B = k_1 (W_s - B) \quad \text{(Eq. 8)}$$

Thus, Eq. 3 becomes:

$$\frac{dC}{dt} = k_2 B = k_1 (W_s - B) = \frac{-dA}{dt} \quad \text{(Eq. 9)}$$

in which B is the steady-state weight of drug in the aqueous phase. The rate of change of C with time thus becomes constant and is equal to the dissolution rate $-dA/dt$ of the tablets. Division of dC/dt by $(W_s - B)$ yields k_1, while division of dC/dt by B yields k_2. The values of k_1 and k_2, evaluated by the various methods for the data shown in Fig. 1, are given in Table II.

The fact that the rate of appearance of the drug in the lipid phase becomes equal to the dissolution rate of the drug when B reaches a steady state is of great potential value in dissolution rate studies. This would be useful, for example, for studies involving poorly water-soluble drugs which would re-

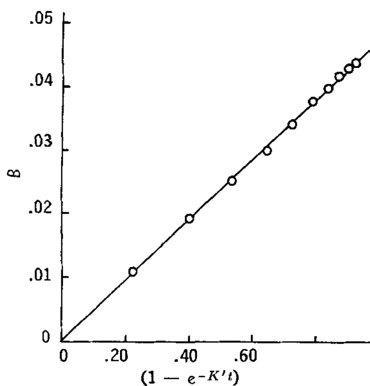


Fig. 5—Plot of Eq. 6 for the appearance of salicylic acid in the pH 2.00 buffer. Stirring rate was 100 r.p.m. at 30°.

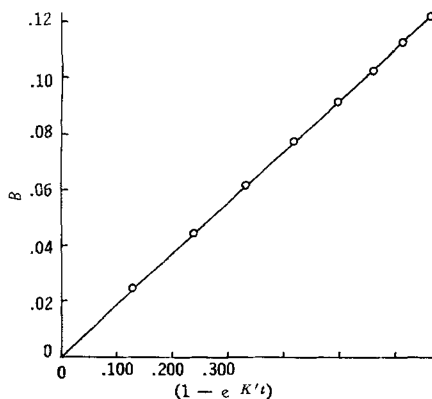


Fig. 6—Plot of Eq. 6 for the appearance of salicylic acid in the 0.1% w/v polysorbate 80 solution at pH 2.00. Stirring rate was 100 r.p.m. at 30°.

Table II—Comparison of k_1 and k_2 Obtained by Various Methods

Constant, min.$^{-1}$	Method Eq. 6	Eq. 4	Eq. 9
k_1	0.00293	0.00292	0.00291
k_2	0.0316	....	0.0314

quire large volumes of dissolution medium to obtain meaningful results. Large, and sometimes unwieldy, volumes of dissolution medium are also used in dissolution rate studies to prevent build up of the drug in the dissolution medium so the assumption of $W_s \gg B$, or its equivalent in concentration terms, can safely be assumed. The presence of the lipid phase acting as a sink, as it did in these studies, would obviate this difficulty. In some instances, the assay for a drug in the aqueous phase might be difficult or perhaps time consuming in that an extraction into a lipid phase is necessary. The ability to measure dissolution rates by analyzing a lipid phase, as done in these studies, would then be of great value.

Effect of Stirring Rate—The effect of stirring rate upon both constants was determined from 50–200 r.p.m. at 30 ± 0.05° using the steady-state equations. The relationship between rate constants and stirring rate may be given by:

$$k = a\,(\text{r.p.m.})^b \qquad \text{(Eq. 10)}$$

in which a and b are constants. The constant b has been found to range from zero for a nondiffusion-

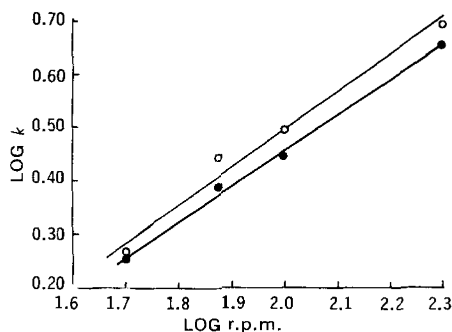


Fig. 7—Effect of stirring rate upon k_1 *and* k_2 *at 30°. Key:* ●, $(\log k_1) + 3$; ○, $(\log k_2) + 2$.

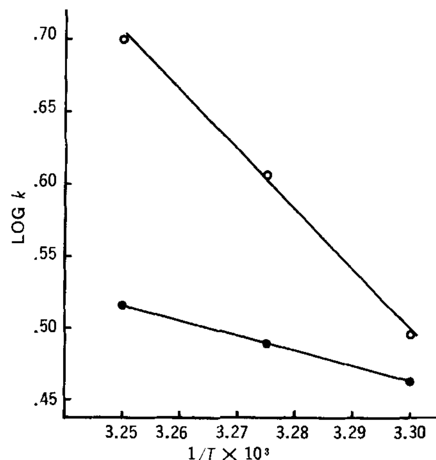


Fig. 8—Arrhenius plots for k_1 *and* k_2 *at a stirring rate of 100 r.p.m. Key:* ●, $(\log k_1) + 3$; ○, $(\log k_2) + 2$.

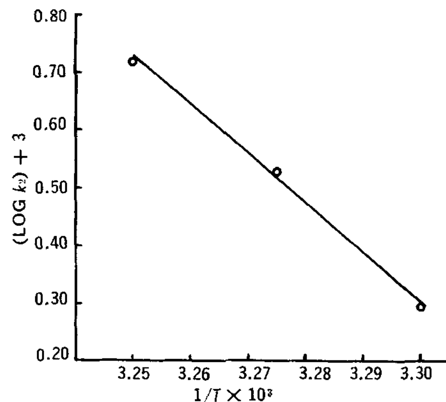


Fig. 9—Arrhenius plot for the effect of temperature upon corrected k_2 *at a stirring rate of 100 r.p.m.*

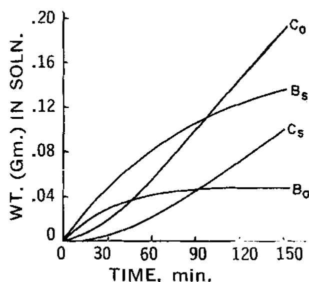


Fig. 10—Effect of polysorbate 80 upon the dissolution and partitioning rates of salicylic acid at a stirring rate of 100 r.p.m. and 30°. Key: B_0 *represents the dissolution into pH 2.00 buffer,* B_s *represents the dissolution into the polysorbate 80 solution,* C_0 *represents the partitioning into octanol in the absence of the polysorbate 80, and* C_s *represents the partitioning into octanol in the presence of polysorbate 80.*

controlled process to approximately one for a diffusion-controlled process (6). Plots of log k *versus* log (r.p.m.) are shown in Fig. 7. The constant, b, was found to be 0.67 and 0.70 for k_1 and k_2, respectively, indicating both processes are diffusion controlled.

Effect of Temperature—The effect of temperature upon both rate constants was determined at 100 r.p.m. through the temperature range of 30–35° ± 0.05° using the steady-state equations. The data are plotted according to the Arrhenius relationship in Fig. 8. The energy term associated with k_1 was found to be 4,690 cal./mole, which is in good agreement with the values of 4,446–4,548 cal./mole reported by Edwards (7) for the energy of diffusion within this temperature range. The energy term associated with k_2, however, was found to be 19,100 cal./mole which would indicate a nondiffusion-controlled process. This is not in agreement with the findings of the stirring rate data. The basic mechanism was therefore modified to include a diffusion process for the transport of solute from the aqueous to the lipid phase:

$$\frac{dC}{dt} = k_2 B (W_c - C) \qquad \text{(Eq. 11)}$$

in which W_c represents the weight of drug needed to saturate the lipid phase. Table I and Figs. 1 and 2 show that for these studies $W_c \gg C$ and:

$$\frac{dC}{dt} = k_2 B W_c = k_2' B \qquad \text{(Eq. 12)}$$

in which $k_2' = k_2 W_c$. This modification would not change the kinetic picture, as given by Eqs. 1–9, or the stirring rate data. It merely indicates that our previously determined k_2 probably contains the term W_c. A plot of the corrected k_2 *versus* temperature is shown in Fig. 9. The energy term associated with the corrected k_2 was found to be 4,640 cal./mole, which is in good agreement with what would be expected for a diffusion-controlled reaction. The modification, as shown by Eq. 11, accounts for dC/dt decreasing as C approaches W_c and for its becoming equal to zero when C becomes equal to W_c. This is more realistic than Eq. 3 which indicates that the transfer of solute into the lipid phase would continue at a constant rate, and does not provide for the eventual saturation of the lipid phase.

Effect of Polysorbate 80[2]—Sufficient polysorbate 80 was added to the pH 2.00 buffer to give a final concentration of 0.20% w/v. The data were obtained in the usual manner for a stirring rate of 100 r.p.m. at 30 ± 0.05°. The results are shown in Fig. 10. The dissolution rate, as shown by the plots of B *versus* time, is indeed increased in the presence of the surfactant, in agreement with the literature (2,3). The partitioning rate, however, as shown by the plots of C *versus* time is decreased in qualitative agreement with the findings of Levy and Reuning (4). Thus, the value of this system for a screening procedure, as opposed to the usual dissolution rate studies, becomes readily apparent. The exact mechanism for the effect of surfactants upon the dissolution and partitioning rates of drugs is currently being studied and will be reported in a future communication.

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[2] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.