Drug Permeation through Thin Model Membranes II: Permeation Characteristics of a Polymeric Model Biomembrane

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Abstract □ A novel synthetic model biomembrane consisting of a polymeric matrix containing several natural membrane constituents has been developed that exhibits permeation characteristics often attributed to natural biological membranes. When used to separate two aqueous phases buffered at physiological pH's, the model membrane constitutes an experimental system simulating gastrointestinal absorption. The transport of salicylic acid from a chamber at pH 2.0 (*A*) through the synthetic membrane and into a receiving chamber at pH 7.4 (*C*) follows apparent first-order kinetics. Support is given to a proposed kinetic model of $A \rightarrow C$ because of negligible membrane retention and minimal back-transport of the test drug. The rate of disappearance from *A* approximated the rate of appearance in *C*. The model system more closely simulates *in vivo* conditions than previously used three-phase liquid *in vitro* models. The pH-partition hypothesis is examined using this experimental system, the results indicating that the unionized, lipid-soluble species is the only form of the drug to permeate the synthetic biomembrane readily.

Keyphrases □ Biomembrane, polymeric—permeation characteristics □ Salicylic acid transport—model biomembrane □ pH-partition hypothesis–salicylic acid absorption—correlation □ UV spectrophotometry—analysis

There is continuing interest in model membrane transport phenomena. Thus, Lieb and Stein (1), working with nonelectrolyte transport across several polymeric films, related diffusion across these homogeneous networks to diffusion across biological barriers. Other investigators (2) impregnated several polymeric materials with various liquid lipoidal materials and used these membranes in a combination dissolution-partition apparatus. More recently, Bates *et al.* (3) used Millipore membrane filters impregnated with olive oil to study the effect of caffeine complexation on transport. Bimolecular (bilayer) lipid membranes have also been subjected to permeation studies employing organic solutes (4).

In a previous report (5), developmental aspects of a thin polymeric model biomembrane were presented. Salicylic acid was used as the test drug, and the apparent first-order disappearance rate constant from a pH 2.0 to a pH 7.4 aqueous environment, through the membrane, was used to assess the permeation capabilities of the membrane. A standard membrane consisting of 44% ethylcellulose, 44% total biological materials, and 12% mineral oil on a dry-weight basis was adopted. This composition afforded a compromise between membrane pliability, stability, and rate of drug transport. The absence of solvent flux through this membrane was also demonstrated, indicating the absence of pores in the polymer structure.

The present communication details some permeation characteristics of the membrane, namely: (*a*) the equivalence of the apparent first-order disappearance and appearance rate constants, k_d and k_a, respectively; (*b*) the concentration independence of the disappearance rate constant; (*c*) the extent of drug retention and the absence of dimerization within the membrane; and (*d*) the adherence to the pH-partition hypothesis of unionized drug absorption.

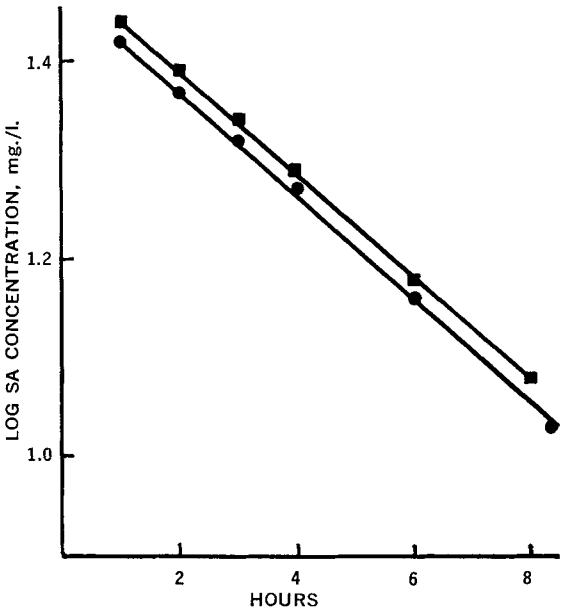

Figure 1—*Semilogarithmic plot of the disappearance and appearance of salicylic acid across the standard model biomembrane, pH 2.0–7.4. Key: ●, disappearance; and ■, appearance.*

EXPERIMENTAL

Materials—The materials used were reported previously (5) with the exceptions of reagent grade citric acid, sodium citrate, and sodium chloride, used as received.

General Experimental Protocol—The procedures for membrane formation, permeation studies using Plexiglas dialysis cells, and salicylic acid determination are as published (5) and remain unchanged, unless noted. All experiments were carried out at 37° using 0.1 *M* constant ionic strength buffers.

Partition Studies—A membrane (cross sectional area of 11.34 cm.^{2}) was placed into a flask containing 75 ml. of a solution of salicylic acid in a pH 2.0 buffer. After 3 hr. of agitation, which was sufficient for equilibrium concentrations to be obtained, the membrane was removed from the drug-containing buffer and the drug content of the buffer was determined spectrophotometrically. Four salicylic acid concentrations in pH 2.0 buffer (323, 422, 634, and 787 mg./l.) and one in pH 7.4 buffer (294 mg./l.) were employed. All determinations were made in triplicate.

Compliance with the pH-Partition Hypothesis—The buffer solutions used were 0.1 *N* HCl (pH 1.2), a hydrochloric acid buffer (pH 2.0), citric acid and sodium citrate (pH 3.0 and pH 4.0), and a phosphate buffer (pH 7.4). Salicylic acid (pKa = 3.0) determinations in pH 1.2, 3.0, and 4.0 buffers were made at 303, 301, and 298 nm., respectively, the wavelength of maximum absorbance. The duration of each experimental run was well past one half-life, with the exceptions of pH 4.0 and 7.4, which were followed for a period of 44 ($t_{42\%}$) and 68 hr., respectively. The apparent first-order disappearance-rate constant, k_d, was converted to the true first-order disappearance-rate constant, k_d', by correcting for the

Table I—Effect of Initial Salicylic Acid Concentration on the Disappearance-Rate Constant from pH 2.0 Compartment

Initial Concentration of Salicylic Acid, mg./l	Apparent Disappearance-Rate Constant, $k_d \times 10^3$ (hr.$^{-1}$)
30.8	117
300	123
772	122

relative amount of unionized drug species present in the system using the following equation:

$$k_d' = k_d/f \qquad \text{(Eq. 1)}$$

where f is the fraction of unionized salicylic acid.

RESULTS AND DISCUSSION

Equivalence of k_d and k_a—Figure 1 depicts the results of salicylic acid permeation through the model membrane within the *in vitro* transport system. The lower line represents the disappearance of salicylic acid from the pH 2.0 chamber, corrected for the equilibrium concentration of this chamber. The upper line denotes the appearance of salicylic acid in the pH 7.4 receiving chamber. This line is constructed by considering the equilibrium concentration in the receiving chamber and subtracting from it the observed concentrations in the chamber. Essentially all of the salicylic acid was transported, thereby allowing the equilibrium concentration in the receiving chamber to be taken as C_A^0 (Eq. 3) and the equilibrium concentration in the initial chamber to be considered as zero. Figure 1 is presented in this fashion to demonstrate the parallelism observed; that is, the equivalence of the apparent first-order rate constants, k_d and k_a, obtained from this plot. By using the method of least squares, these constants were derived from sampling periods after the attainment of steady-state conditions (which appears to be a rapid process) and were shown to be experimentally identical. From these plots, the amount of salicylic acid in the membrane under steady-state condition was computed to be 3.14% of the initial concentration.

Diffusion may be quantitated in terms of Fick's first law, which in turn may be modified when considering transport through a barrier quite thin in cross sectional dimension. Upon rearrangement and term collection, a simple first-order rate expression may be derived, the integrated form of which is characteristically plotted when first-order transport kinetics are suspected. These expressions appear as Eqs. 2 and 3, respectively, where: C_A^0 is the initial concentration in Compartment A, which represents the lumen of the gastrointestinal tract; C_A is the drug concentration within the same compartment at time equal to t; and k_d is as previously defined. Thus,

$$-\frac{dC_A}{dt} = k_d C_A \qquad \text{(Eq. 2}$$

$$\log C_A = \log C_A^0 - \frac{k_d t}{2.303} \qquad \text{(Eq. 3)}$$

From Eq. 3, a plot of log C_A *versus* t should yield a straight line, from the slope of which is obtained the value of k_d. Figure 1 is such a representation. The use of Fick's law as a basis for these passive diffusion equations requires that two assumptions be made: (*a*) that the barrier is thin and hence solute content is small, and (*b*) that a single rate constant expresses the material flux. Essentially, this describes a two-compartment transport model.

The first assumption is fulfilled by the negligible salicylic acid retention within the model membrane, also demonstrated by partition studies. Although k_d appears to be a hybrid rate constant reflecting a reversible exchange of unionized drug with Compartment A, the large partition coefficient in favor of the membrane phase indicates the exchange is essentially unidirectional. Regardless of the complexity of the process, k_d is experimentally equivalent to k_a, thereby satisfying the second assumption. Accordingly, Eq. 4, depicting overall unidirectional transport expressed by a single rate constant, best reflects the kinetics of this *in vitro* transport system:

$$A \xrightarrow{k_d \text{ (or } k_a)} C \qquad \text{(Eq. 4)}$$

Table II—Partition of Salicylic Acid between pH 2.0 Buffer and the Standard Model Biomembrane

Total Salicylic Acid in System, mg.[a]	Salicylic Acid in Membrane, mg.[b]	Salicylic Acid in Membrane, %	Partition Coefficient, k_p[c]
24.25	0.54	2.23	75.6
31.62	0.65	2.06	69.7
47.57	1.05	2.21	74.9
59.05	1.21	2.05	69.4
22.04[d]	Nil	Nil	—

[a] Volume of aqueous phase = 75 ml. [b] Volume of membrane = 22.6×10^{-3} ml. [c] k_p = mg./ml. in membrane/mg./ml. in aqueous phase. [d] Between pH 7.4 and membrane.

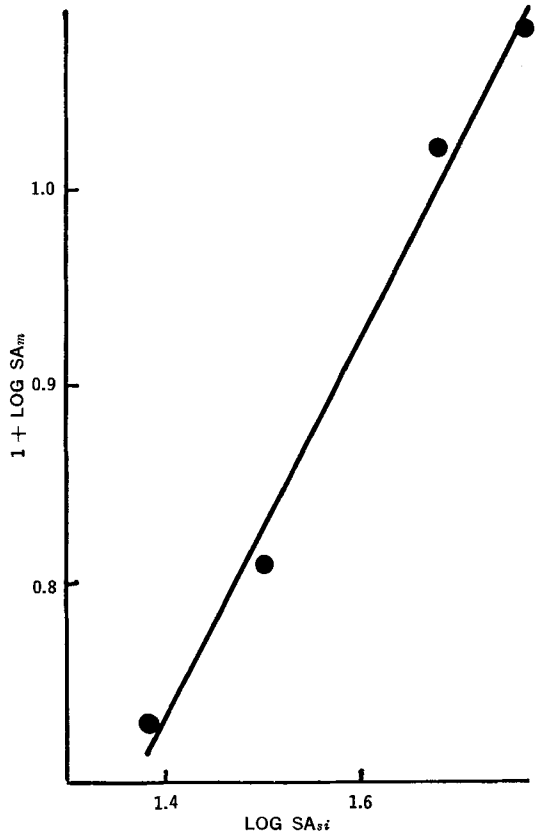

Figure 2—*Log–log plot of salicylic acid in the membrane* (SA_m) versus *salicylic acid initially present in the system* (SA_{si}).

Effect of Initial Concentration on k_d—A series of salicylic acid solutions in pH 2.0 buffer covering a 25-fold concentration range was employed to study the effect of initial concentration on the disappearance-rate constant across the model membrane. The results are contained in Table I; the right-hand column suggests that k_d is constant over the concentration range examined, *i.e.*, characteristic of first-order rate processes.

Partition Studies—Partition studies were undertaken to clarify the extent of solute association and the percent solute retention within the membrane. Initial salicylic acid concentrations were noted and again determined after 3 hr. in the buffer. As Table II indicates, both the amount of drug within the membrane and the percent of the total amount within the membrane were computed. The average percent retention of salicylic acid was 2.14% and is of the same magnitude as the value obtained from the permeation studies. The percent retention is also presented as an apparent partition coefficient, here defined as the ratio of drug/milliliter in the membrane to drug/milliliter in the aqueous environment. The results obtained from the pH 7.4 buffer demonstrate that the

Table III—Effect of pH on Disappearance-Rate Constant of Salicylic Acid Using the Standard Model Biomembrane

pH of Initial Compartment	Apparent Disappearance-Rate Constant, $k_d \times 10^3$ (hr.$^{-1}$)	Unionized Salicylic Acid, %	True Disappearance-Rate Constant, $k_d' \times 10^3$ (hr.$^{-1}$)[a]
1.2	139	98.4	141
2.0	123	90.9	135
3.0	69.5	50.0	139
4.0	12.4	9.1	136
7.4	Nil	0.0	Nil

[a] See Eq. 1.

ionized form of salicylic acid is unable to enter the model membrane.

Of interest, when considering the actual mechanism of permeation within the solid polymeric barrier, is the phenomenon of intermolecular associations of the penetrant molecules and their effects on the observed rate constants. Gonzales *et al.* (6), studying the diffusion and solubility of various compounds through polyethylene, observed that both benzyl alcohol and benzoic acid had the lowest diffusion of the test compounds. They attributed this observation to the potential of these compounds to exhibit intermolecular hydrogen bonding, thereby forming dimers and trimers whose diffusion within the polymer was thought to be restricted. From the partition data in Table II and Eq. 5, it is possible to demonstrate whether or not this phenomenon is operative in the standard model biomembrane. Thus,

$$\log SA_m = \log SA_{si}/n + \log MCR/n \qquad \text{(Eq. 5)}$$

where *MCR*, the membrane content ratio, is the ratio of drug in the membrane to the drug initially present in the system; SA_m and SA_{si} are the amounts of salicylic acid within the membrane and initially present in the system, respectively; and n is the degree of association of salicylic acid in the membrane. From Eq. 5 a log–log plot of SA_m *versus* SA_{si} should yield a straight line, the slope of which is $1/n$. If n assumes the value of unity, it may be assumed that dimer and trimer formation of salicylic acid is absent in this experimental system. Figure 2 illustrates such a plot, and the value of n was found to be 1.04. Hence, it is reasonable to assume the absence of solute interaction in the polymeric barrier.

Membrane Compliance with the pH-Partition Hypothesis—To establish whether or not the model membrane simulated biological membrane functionality with regard to passive drug absorption, an evaluation of the pH-partition hypothesis (7–11) was conducted (Table III). The apparent first-order disappearance-rate constants for the five pH values examined were converted to true first-order disappearance-rate constants by correcting for the amount of unionized drug present. The consistency of these data and the absence of any detectable transfer at pH 7.4 strongly suggest that only the unionized form of the drug crosses the barrier. Accordingly, these results are in good agreement with the predictions made by the pH-partition hypothesis relating the biological membrane to a nonpolar barrier which permits only nonpolar substances to cross readily.

SUMMARY

As a result of the low retentive qualities of the membrane and the equivalence of the rate constant for disappearance from Compartment A (k_d) and the rate constant for appearance in Compartment C (k_a), a two-compartment absorption model has been shown to fit the experimental system. The observed lack of concentration influence on k_d is consistent with first-order kinetics. A lack of molecular association within the membrane has been demonstrated, and the model membrane has been shown to adhere to the pH-partition hypothesis. Attempts to correlate *in vitro* drug permeation to *in vivo* drug absorption are currently in progress and will be the subject of future reports.

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