Effect of Lipid Polarity and Cell Design on the *In Vitro* Transport of Salicylic Acid

MATTHEW A. AUGUSTINE and JAMES SWARBRICK

Abstract □ The transport kinetics of salicylic acid have been studied in a Schulman-type *in vitro* model cell containing two aqueous phases (*A* and *C*) separated by a third liquid lipid phase (*B*) simulating the biomembrane. Prime consideration was given to evaluating the effect of increasing the polarity of the lipid phase on the two forward rate constants, K_1 and K_2. While K_1 ($A \rightarrow B$) always increased with increasing polarity of the lipid phase, K_2 ($B \rightarrow C$) was found to increase or decrease depending on the stirring conditions employed in the two aqueous phases. The surface-to-volume ratio of the immiscible phases was significant in determining the magnitude of the rate constant K_1.

Keyphrases □ Salicylic acid—*in vitro* transport kinetics □ Lipid phase polarity effect—salicylic acid transport □ Surface-to-volume ratio, stirring—salicylic acid transport □ Schulman cell, evaluation—transport studies □ Partition coefficients, salicylic acid—oil-water phases

With model *in vitro* systems that employ liquid lipids to simulate the biomembrane, there is the possibility that drug concentration may build up in the lipid phase. Such an effect is in contrast to the *in vivo* situation and reduces the validity of the *in vitro* model.

The retention of drug in the lipid phase will be, in part, a function of the polarity of the lipid and this, in turn, will affect the rate constants which describe the transport of the drug through the model system. Such an effect was demonstrated by Khalil and Martin (1) who used the inverted Y-tube as an *in vitro* model. Using various single-component lipid materials as the "membrane" phase, these workers obtained forward and reverse rate constants for the transport of salicylic acid: (*a*) from an aqueous pH 2.0 phase to the lipid phase (K_1 and K_{-1}, respectively), and (*b*) from the lipid phase to a second aqueous phase buffered to pH 7.4 (K_2 and K_{-2}, respectively). It was observed, predictably, that as the polarity of the lipid phase, expressed in terms of the δ-value (2), increased, the forward rate constant K_1 increased while the forward rate constant K_2 decreased.

The *in vitro* model cell designed by Schulman (3) has been claimed to possess certain advantages over the inverted Y-tube (4). Thus, long-chain alcohols can be used as the lipid phase with less chance of emulsification; phospholipid material can also be placed at the oil-water interfaces which do not continuously expand and contract in area, as is the case with the inverted Y-tube. Accordingly, the Schulman-type cell was chosen for these *in vitro* transport studies.

As a preliminary study, the effect of polarity of the lipid phase on the transport kinetics of salicylic acid was investigated. The polarity of the oil phase was increased by the addition of isoamyl alcohol to cyclohexane, with the expectation of finding at least a qualitative correlation between these data and that of Khalil and Martin. The results obtained demonstrate, however, that the magnitude and rank order of the rate constants are dependent to a significant degree on the design of the cell and the agitation conditions used.

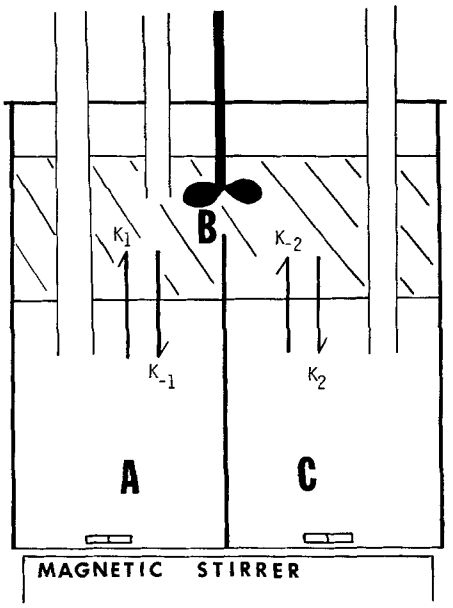

Figure 1—*Schulman-type* in vitro *model cell. See text for explanation of symbols.*

EXPERIMENTAL

***In Vitro* Model Cells**—Three Schulman-type cells, designated I, II, and III, similar to that shown in Fig. 1 were used in these preliminary studies. These model cells, constructed of Plexiglas (I, II) or glass (III), physically simulate the two membrane interfaces found *in vivo*, one between the gastrointestinal fluid (*A*) and the membrane (*B*) and one between the membrane and blood plasma (*C*). Either gastric or intestinal absorption can be studied by choosing the appropriate pH conditions for Compartment *A*, while keeping the pH of Compartment *C* constant at 7.4.

The two aqueous compartments were stirred using Teflon-coated magnetic stirring bars, and the oil phase was stirred using an overhead constant-speed motor equipped with shaft and propeller (see Tables I and II). The cells were maintained at 25 ± 0.1° in all the studies performed.

The volumes of liquid used for each of the three phases in the various cells are shown in Tables I and II. Also included in these tables are the surface-to-volume ratios of the cells, which are based on the volume of either aqueous phase and the surface area in contact with the lipid phase, *B*, in the absence of any agitation. Only slight rippling of the interfaces was noted with the various agitation conditions employed.

Materials—Salicylic acid, cyclohexane, isoamyl alcohol, and the buffer ingredients were all reagent grade. Clark and Lubs buffer solutions at pH 2.0 and pH 7.4 were prepared and their pH values checked prior to use.[1]

Procedure—In all studies, Compartment *A* was maintained at pH 2.0 while the receiving compartment, *C*, was buffered to pH 7.4.

[1] Beckman Expandomatic pH meter, Beckman Instruments, Inc., Fullerton, Calif.

Table I—Solubility, Partition Coefficients, and Forward Rate Constants for Salicylic Acid

Isoamyl Alcohol in Cyclohexane, % v/v	Solubility, g./100 ml.	Apparent Partition Coefficient[a] Oil/pH 2.0	Apparent Partition Coefficient[a] pH 7.4/Oil	Forward Rate Constants ($hr.^{-1}$)[b] K_1	Forward Rate Constants ($hr.^{-1}$)[b] K_2
5	1.74	11.0 (±0.2)	994 (±140)	0.320	0.115
10	3.13	22.1 (±0.3)	558 (±83)	0.430	0.213
20	5.58	43.7 (±1.5)	274 (±12)	0.520	0.281
30	7.96	65.9 (±6.0)	167 (±16)	0.650	0.410

[a] Average of three initial salicylic acid concentrations, 20, 40, and 50 mg. per 20 ml. (±*SD*). [b] Cell I, rectangular Plexiglas; volume of each phase is 550 ml.; surface-to-volume ratio is 0.124; aqueous phases stirred at 300 r.p.m. with 2.54-cm. (1-in.) magnetic stirring bar; oil phase stirred at 60 r.p.m.

Table II—Effect of the Surface-to-Volume Ratio and Stirring Conditions on Salicylic Acid Transport

Cell	Surface-to-Volume Ratio	Stirring Conditions	% (v/v) Isoamyl Alcohol in Cyclohexane	Forward Rate Constants ($hr.^{-1}$) K_1	Forward Rate Constants ($hr.^{-1}$) K_2
II[a]	0.148	300 r.p.m. with 2.54-cm. (1-in.) magnets	10	0.650	0.174
			20	0.752	0.202
			30	0.885	0.400
II	0.148	400 r.p.m. with 1.27-cm. (0.5-in.) circular magnets	10	0.500	0.106
			20	0.602	0.105
			30	0.652	0.089
III[b]	0.180	300 r.p.m. with 2.54-cm. (1-in.) magnets	10	0.520	0.120
			20	1.100	0.360
			30	1.250	0.410

[a] Circular glass cell; volume of each phase = 225 ml.; oil phase stirred at 60 r.p.m. [b] Rectangular Plexiglas cell; volume of each phase = 250 ml.; oil phase stirred at 60 r.p.m.

The two aqueous phases were preequilibrated with respect to the lipid phase prior to the transport studies. At various time intervals following the addition of salicylic acid into Compartment *A*, samples were removed from the aqueous and lipid phases by pipet and assayed for salicylic acid by means of previously prepared calibration curves. In all studies, Beer's law was found to be obeyed over the concentration range examined. The volume of each of the sampled phases was kept constant by replacing a quantity of either buffer or oil equal to that removed for assay. Calculations were based on the percent of salicylic acid remaining in each of the phases with corrections made for the quantity of salicylic acid removed with each sample.

Analysis of Data—The kinetics governing *in vitro* drug transport in a three-phase system have been described elsewhere (1). The solution of the differential equations involved in a consecutive reversible first-order system requires the calculation of four rate constants. These are the two forward rate constants, K_1 and K_2, which govern the transport of drug between Compartments *A* and *B* and *B* and *C*, respectively, and their corresponding reverse rate constants, K_{-1} and K_{-2}. To facilitate their determination, an analog computer[2] was employed whose output was displayed on an X-Y recorder. The analog program and the differential equations are shown in Fig. 2. The volume terms associated with the solution of these equations are absent since the volumes of all three phases were kept equal. Once goodness-of-fit of the experimental and theoretical curves was achieved with the analog computer, the resultant rate constants were then verified using a digital computer[3] programmed for the integrated forms of the differential equations (5).

Solubility of Salicylic Acid—An excess of salicylic acid was placed in 100 ml. of each of the various lipid phases contained in 125-ml. ground-glass-stoppered flasks. The flasks were shaken in a constant-temperature shaker bath for 2 weeks at 25°. Filtered, diluted samples were assayed spectrophotometrically[4] for salicylic acid, using previously determined Beer's law plots.

Determination of Partition Coefficients—Twenty milliliters of either pH 2.0 or pH 7.4 aqueous buffers containing a known amount of salicylic acid was shaken in a 60-ml. separator with an equal volume of the various lipid phases used in the transport studies. The separators were placed in a constant-temperature bath at 25° and shaken manually until equilibrium was attained. Both phases were assayed spectrophotometrically for salicylic acid and the apparent partition coefficients calculated.

RESULTS AND DISCUSSION

The percents of salicylic acid in each of the three phases at various time intervals and isoamyl alcohol concentrations are shown in Figs. 3–5 for Cell I. The disappearance of salicylic acid from the compartment buffered at pH 2.0 is plotted against time in Fig. 3. As the polarity of the lipid phase is increased by the addition of isoamyl alcohol in cyclohexane, the rate of disappearance of salicylic acid increases. Consequently, the initial rate of appearance of

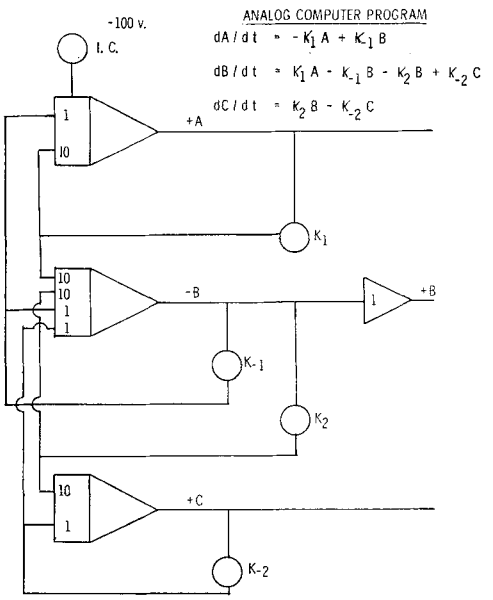

Figure 2—*Differential equations used for consecutive reversible first-order kinetics and analog computer program.*

[2] PACE 261R, EAI, Variplotter 1100, EAI, Long Island, N. Y.
[3] IBM model 360/65.
[4] Beckman model DB-G spectrophotometer, Beckman Instruments, Inc., Fullerton, Calif.

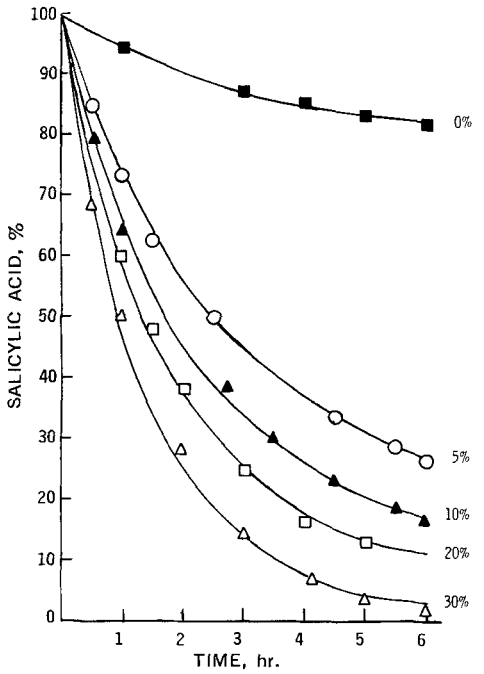

Figure 3—*Rate of disappearance of salicylic acid from Compartment* A, *buffered at pH 2.0, to Compartment* B, *composed of increasing amounts of isoamyl alcohol in cyclohexane.*

salicylic acid in the oil phase increases with increasing polarity of this phase (Fig. 4). However, while the time for the peak concentration of drug to appear in the oil phase was progressively reduced as this phase became more polar, the concentration of salicylic acid at the peak was not directly related to the polarity. The appearance of salicylic acid in the pH 7.4 buffer is shown in Fig. 5. As the polarity of the oil phase increases, so does the rate of appearance of salicylic acid into this phase.

In contrast to the results of Khalil and Martin (1), both forward rate constants, K_1 and K_2, for salicylic acid in Cell I were found to increase as the polarity of the oil phase increased. These data are presented in Table I and Fig. 6. The rank order of the K_1 values obtained with Cell I was in agreement with those previously reported. However, the reverse was apparently true in the case of the K_2 values, which increased with increased polarity of the oil phase.

In an attempt to rationalize these results, the solubility of salicylic acid in each of the various lipid phases was determined to see if saturation of this phase was occurring. While it was found that the solubility of salicylic acid increased linearly with increasing concentrations of isoamyl alcohol in cyclohexane (Table I), the concentration of salicylic acid employed in the transport studies (3.63 mg./100 ml.) was far below that needed to saturate the oil phase.

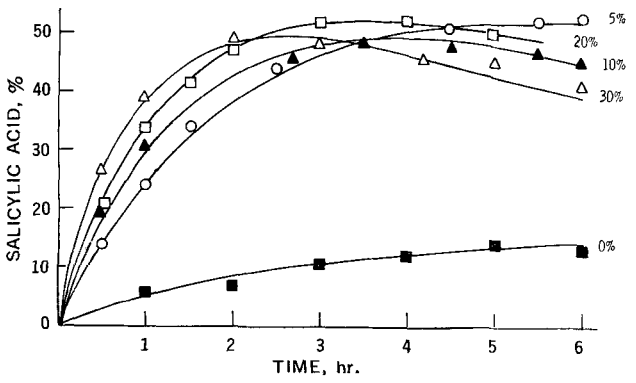

Figure 4—*Initial rate of appearance of salicylic acid in Compartment* B *as a function of the amount of isoamyl alcohol in cyclohexane.*

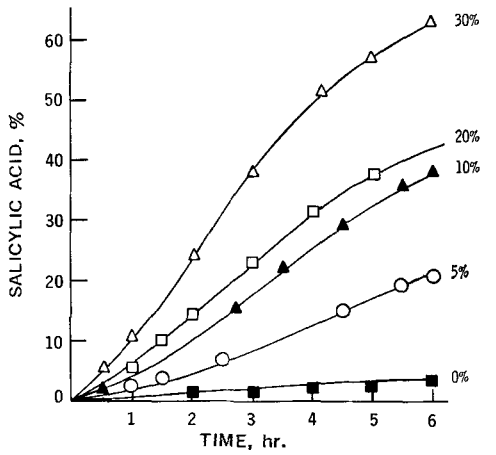

Figure 5—*Rate of appearance of salicylic acid in Compartment* C, *buffered at pH 7.4, as a function of the composition of the lipid phase.*

The next parameter investigated was the equilibrium partitioning of salicylic acid between the two aqueous phases and the various oil phases. The apparent partition coefficients were calculated based on the concentration of drug in each of the two phases at equilibrium. The possibility of dimerization of salicylic acid in the oil phase was also investigated by using initial concentrations of 20, 40, and 50 mg. of salicylic acid per 20 ml. of buffer solution. However, the data presented in Table I did not indicate the presence of a dimer in the lipid phase. As would be expected, both partition coefficients vary in a manner that is proportional to the concentration of isoamyl alcohol in the lipid phase. The relatively high standard deviations obtained for the pH 7.4/oil systems are due to the very low concentrations of salicylic acid remaining in the oil phase at equilibrium.

Although the ratio of the partition coefficient (oil/pH 2.0) to K_1 does not remain constant as the concentration of isoamyl alcohol is increased, K_1 does increase with increasing partition coefficient, as would be expected.

Predictably, the apparent partition coefficients based on the concentration of salicylic acid in the pH 7.4 buffer over that in the various lipid phases (pH 7.4/oil) were found to decrease as the polarity of the lipid phase increased. However, when these apparent partition coefficients were compared to K_2, the reverse of what would be expected, based on the partition data, occurred (Table I). Thus, the forward rate constant K_2 would be expected to decrease with increasing polarity of the lipid phase. As noted earlier, the values obtained for K_2 with Cell I increased with polarity.

Further transport studies were then carried out at 300 r.p.m. with 2.54-cm. (1-in.) magnetic stirring bars in the aqueous phases using two other model cells having surface-to-volume ratios different from that of Cell I (Tables I and II). Within any one cell, the rank order of

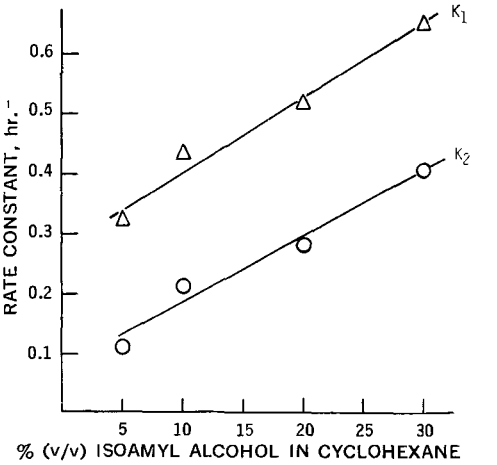

Figure 6—*The effect of increasing lipid polarity of the oil phase on the two forward rate constants,* K_1 *and* K_2, *for salicylic acid.*

K_1 with increasing polarity of the lipid phase was not changed. However, the data demonstrate a relationship between the surface-to-volume ratio and K_1. Thus, with one exception (Cell II, 10% isoamyl alcohol), the value of K_1 increased as did the surface-to-volume ratio for any one lipid phase employed. Under the same stirring conditions of the aqueous phases [namely, 300 r.p.m. with 2.54-cm. (1-in.) stirring bars] in any one cell, K_2 still showed increasing values with increasing lipid polarity. There was no rank order correlation between the value of K_2 for a particular lipid phase and the surface-to-volume ratio.

The effect of stirring conditions in the aqueous phases was then investigated. While keeping the surface-to-volume ratio constant at 0.148, the stirring conditions of the aqueous compartments were arbitrarily changed from 300 r.p.m. using 2.54-cm. (1-in.) oblong magnetic stirring bars to 400 r.p.m. using 1.27-cm. (0.5-in.) circular magnetic stirring bars. The lipid phase was stirred at the same rate of 60 r.p.m. The data, which were obtained using Cell II, are presented in Table II. It is readily apparent that the size and revolutions per minute of the stirrer did not change the order of the K_1 values, although the magnitude of this rate constant was affected. What is relevant is that, under the second set of stirring conditions, the order of K_2 now is reversed, with K_2 tending to decrease with increasing lipid polarity, as found by Khalil and Martin (1).

In light of these preliminary results, it would appear that the use of the Schulman model cell to correlate *in vitro* with *in vivo* absorption data could produce misleading results if factors such as the size and shape of the stirring bars and the rate of stirring in the various compartments are not closely monitored. The authors have also found further discrepancies between the Schulman cell and the inverted Y-tube apparatus. For example, benzene, when used as the lipid phase by Khalil and Martin, appeared to be ideal, because there was negligible retention of salicylic acid in this phase over a 24-hr. period. However, when benzene was used as the lipid phase in Cell II, appreciable retention of salicylic acid by this phase was observed over the same time period.

REFERENCES

(1) S. A. Khalil and A. N. Martin, *J. Pharm. Sci.*, **56**, 1225(1967).

(2) J. H. Hildebrand and R. L. Scott, in "The Solubility of Nonelectrolytes," 3rd ed., ACS Monograph No. 17, Reinhold, New York, N. Y., 1950.

(3) A. M. Agostini and J. H. Schulman, in "Surface Activity and the Microbial Cell," Gordon and Breach, New York, N. Y., 1965, p. 37.

(4) J. Perrin, *J. Pharm. Pharmacol.*, **19**, 25(1967).

(5) A. A. Frost and R. G. Pearson, in "Kinetics and Mechanisms," 2nd ed., Wiley, New York, N. Y., 1961, p. 176.

ACKNOWLEDGMENTS AND ADDRESSES

Received July 14, 1969, from the *Division of Pharmaceutics, School of Pharmacy, University of Connecticut, Storrs, CT 06268*

Accepted for publication September 22, 1969.

Presented to the Basic Pharmaceutics Section, APhA, Academy of Pharmaceutical Sciences, Montreal meeting, May 1969.

Supported by a Warner-Lambert Research Fellowship (M.A.A.) and a grant from the University of Connecticut Research Foundation.

Effect of pH of Precipitation on Antacid Properties of Hydrous Aluminum Oxide

STANLEY L. HEM*, EMANUEL J. RUSSO, SURENDRA M. BAHAL, and RALPH S. LEVI

Abstract □ The effect of the pH of precipitation on the physical and chemical properties of hydrous aluminum oxide prepared by the reaction of aluminum chloride and strong ammonia solution NF; aluminum sulfate USP, and strong ammonia solution NF; and aluminum chloride, sodium bicarbonate USP, and sodium carbonate USP, was studied. During aging, changes may occur in the hydrous aluminum oxide structure which result in a loss in acid reactivity. This loss followed apparent first-order kinetics. The rate of loss was directly dependent on the pH of precipitation and continued until a constant end-point was reached. The percentage of theoretical reactivity remaining at the end-point was inversely related to the pH of precipitation. X-ray diffraction showed no differences in form, either initially or during aging. Gel stability appears to depend on the presence of anions in the gel structure. The concentration of these anions is related to the pH of precipitation. Data are presented which demonstrate that a stable, completely acid-reactive gel would be obtained if 1 mole of a monovalent anion such as chloride or bicarbonate or 0.5 mole of a bivalent anion such as sulfate is incorporated in the gel structure per mole of aluminum.

Keyphrases □ Hydrous aluminum oxide—antacid properties, preparation □ pH precipitation effect—hydrous aluminum oxide, chemical and physical properties □ Gel stability—anion concentration data, acid reactivity kinetics □ X-ray diffraction—analysis

Alumina gel, which can be described chemically as hydrous aluminum oxide, is widely used in the management of peptic ulcer and gastric hyperacidity. Several factors, such as the precipitation temperature (1, 2), the order of addition of the reactants (2), and the concentration of the reactants (2, 3), have been shown to affect the properties of the precipitated gel. Although previous authors (4–6) have suggested that the pH of precipitation influences the crystallinity of the precipitated gel, no workers have directly examined the effect of the pH of precipitation on the acid reactivity of the gel. Therefore, the purpose of the present study was to examine the effect of the pH of precipitation on the antacid properties of hydrous aluminum oxide prepared from several reactant systems.

EXPERIMENTAL

Materials—All chemicals used were either official or reagent grade.

Method of Preparation of Hydrous Aluminum Oxide—A series of hydrous aluminum oxides, to be referred to as chloride-containing gels, were prepared at 25° by the addition of a 13% solution of strong ammonia solution NF to an aqueous 8.5% aluminum chloride heptahydrate solution. The method of Papée *et al.* (4) and the conditions described by Lewis and Taylor (2) were followed to ensure reproducible precipitations. Sufficient ammonia solution was added to control the pH of precipitation. Alumina gels were precipitated at pH 4.8, 6.1, 7.7, and 9.2 and washed with deionized water until the concentration of the chloride ion in the filtrate, as determined by the Volhard method (7), was less than 0.1%. The