Effect of Certain Nonionic Surfactants on the Absorption of Salicylic Acid from Solutions by the Frog, *Rana pipiens*

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Abstract □ Three concentrations each of polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80 were added to dilute buffered solutions of salicylic acid and absorption of the drug by the frog studied by an immersion technique. The more dilute concentrations of polysorbate 20 and polysorbate 40 were almost as effective in increasing absorption as were the higher concentrations of polysorbate 60 and polysorbate 80. The absorption process appears to be first-order, based on the concentration of drug remaining in solution. Statistical comparisons were made. Surface tensions of solutions were determined and dialysis methods were used to detect complexation. The surfactants used definitely influenced absorption. The effect appears to be due to complexation and possibly surface tension lowering.

Keyphrases □ Salicylic acid absorption—polysorbates 20, 40, 60, 80 effects □ Absorption, salicylic acid—frog □ Dialysis—salicylic acid complexation measurement □ UV spectrophotometry—analysis

Surface-active agents, especially the nonionic variety, have found wide use in pharmacy and in numerous other fields. Perhaps the most useful property of these substances is their ability to act as solubilizing agents. Certain of these surfactants have been shown to complex with other drugs such as preservatives (1) and thus to interfere with their effectiveness. An aspect of complex formation currently under investigation but not well understood is the part this phenomenon plays in drug absorption. Complexation in some instances is known to influence absorption (2, 3).

EXPERIMENTAL

An immersion technique using the frog, *Rana pipiens*, was employed as in previous work (4, 5). Briefly this consists of placing the frog in 500 ml. of drug solution and assaying the solution every 20 min. for loss of drug. The solutions were $2.5 \times 10^{-4} M$ in salicylic acid and concentrations were determined by reading absorbance at 297 mμ on a spectrophotometer (Beckman DU). Samples were returned to the solution immediately to maintain volume. The polysorbates[1] which were used in concentrations of 0.1, 0.005, and 0.001% did not interfere with the assay of salicylic acid. Ten frogs were used in each determination. The buffer was 0.05 *M* glycine adjusted to a pH of 4.0 with hydrochloric acid. The surface tension of each solution was measured with a surface tensiometer (Fisher).

The degree of complexation of salicylic acid was determined by dialysis (1). Nylon bags[2] were found to be impermeable to the surfactants and the complex but permitted passage of salicylic acid molecules. Twenty milliliters of buffered salicylic acid solution were placed inside the bag which was immersed in 50 ml. of buffered solution of the polysorbate and covered to prevent evaporation. The containers were equilibrated for four days at 30° and concentrations on both sides of the membranes checked. Since the complex absorbs at 297 mμ as does the free salicylic acid which is present in equal concentration on both sides of the membrane, the percent of salicylic acid complexed by each concentration of each polysorbate can be calculated by:

$$\frac{C_o - C_i}{C_o} \times 100$$

where C_o = concentration of complex + concentration of free salicylic acid outside the bag, and C_i = concentration of free salicylic acid on both sides of membrane.

[1] Tweens 20, 40, 60, 80, Atlas Chemical Co. Wilmington, Del.
[2] Tomac, American Hospital Supply.

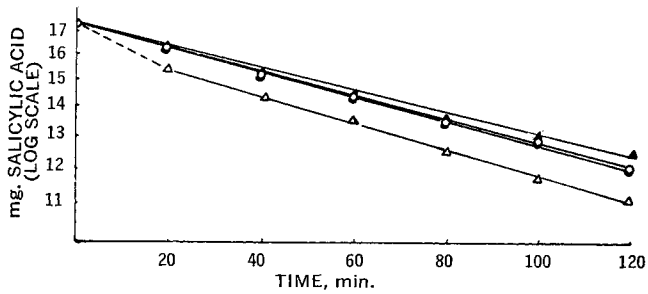

Figure 1—*Effect of polysorbate 20 on amount of salicylic acid remaining in solution over 2-hr. period when 10 frogs each where placed in 500 ml. of aqueous buffered drug solution. Key: ○, salicylic acid, 2.5×10^{-4} M and salicylic acid 2.5×10^{-4} M in presence of polysorbate 20 in concentrations of: △, 0.001%; ●, 0.005%; and ▲, 0.1%.*

RESULTS

As seen in Figs. 1–4 the data can be linearized satisfactorily by plotting the log of amount of drug remaining in solution against time. In some instances the relationship does not become linear until after the initial 20-min. period.

The linear equation is:

$$\log C = -k/2.303t + \log C_o$$

where C = concentration of salicylic acid remaining in solution in mg./500 ml. of solution, C_o = initial concentration of salicylic acid in mg./500 ml. of solution, k = rate constant, and t = time in minutes.

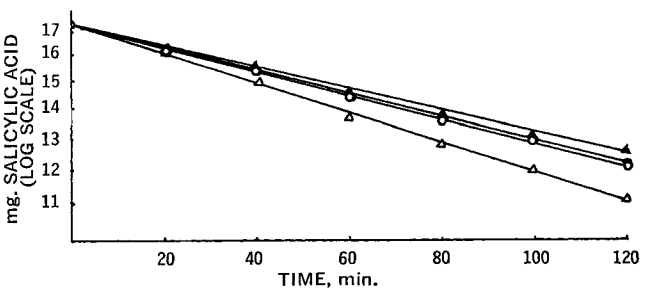

Figure 2—*Effect of polysorbate 40 on amount of salicylic acid remaining in solution over 2-hr. period when 10 frogs each were placed in 500 ml. of aqueous buffered drug solution. Key: ○, salicylic acid, 2.5×10^{-4} M and salicylic acid 2.5×10^{-4} M in presence of polysorbate 40 in concentrations of: △, 0.001%; ●, 0.005%; and ▲, 0.1%.*

Table I Surfactant-Salicylic Acid rate constants

Surfactant	% S.A. Complexed	Rate Constant $min.^{-1} \times 10^5$	Surface Tension, dynes/cm.
Polysorbate 20			
0.001%	12.9	274	50.7
0.005%	7.1	292	43.3
0.1 %	8.0	283	40.2
Polysorbate 40			
0.001%	5.5	395	50.9
0.005%	4.1	292	45.8
0.1 %	5.4	272	42.1
Polysorbate 60			
0.001%	5.3	375	52.5
0.005%	5.4	344	48.0
0.1 %	4.5	286	43.7
Polysorbate 80			
0.001%	5.9	299	53.0
0.005%	10.0	287	48.3
0.1 %	11.2	315	44.3
Control	—	298	57.0

The rate constants (Table I) may be calculated from the equation or from the slope of the line which is equal to $-k/2.303$.

Figures 1–4 are a plot of the log of concentration of salicylic acid remaining in solution against time. Rate constants in Table I indicate the rate of disappearance of drug from solution. As previously pointed out, in some instances the plots do not become linear until after the initial 20-min. period. Possibly the nonsteady state during this time interval is due to binding of salicylic acid to the biological membrane or to its accumulation in the membrane in addition to absorption of the drug. Since some of the plots represent a nonsteady state, statistical comparisons between control and treated solutions were made using both the rate constants which represent steady state only and the average milligrams of drug remaining in solution at the end of 2 hr.

Polysorbate 20—As seen in Fig. 1, polysorbate 20 increased the absorption of salicylic acid when in a concentration of 0.001%. Statistical comparison using the Student *t* test and comparing the amount of drug remaining in solution in the control and in the treated solutions showed 0.001% polysorbate 20 to significantly increase absorption at slightly above the 90% confidence level. For the 0.005 and 0.1% concentrations the results were not significantly different from the control even though they appear to slightly decrease absorption. The rate constants do not differ significantly from that for the control for any of the three solutions of polysorbate 20.

Table I shows the degree of complexation of polysorbate 20 with salicylic acid in the three concentrations used. It is seen that the most dilute solution of polysorbate 20 which appears to have a tendency to increase absorption showed the greatest degree of complexation with salicylic acid. This unusual result leads to speculation that micelle formation may be influencing both dialysis or complexation and absorption of salicylic although surface tensions shown in Table I do not indicate critical micelle formation.

Polysorbate 40—Similar results were obtained with polysorbate 40

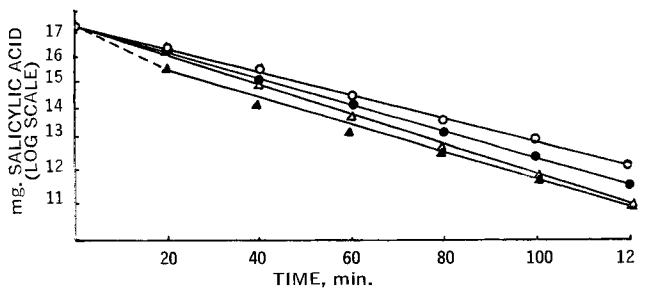

Figure 3—*Effect of polysorbate 60 on amount of salicylic acid remaining in solution over 2-hr. period when 10 frogs each were placed in 500 ml. of aqueous buffered drug solution. Key: ○, salicylic acid, 2.5 × 10⁻⁴ M and salicylic acid 2.5 × 10⁻⁴ M in presence of polysorbate 60 in concentrations of: △, 0.001%; ●, 0.005%; and ▲, 0.1%.*

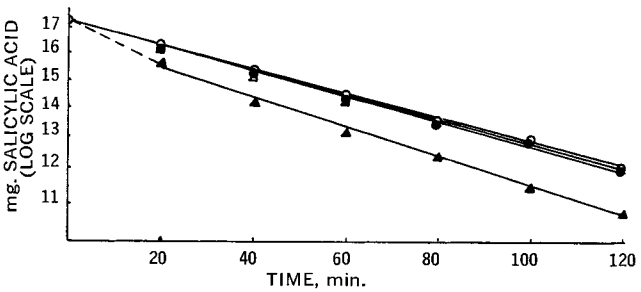

Figure 4—*Effect of polysorbate 80 on amount of salicylic acid remaining in solution over 2-hr. period when 10 frogs each were placed in 500 ml. of aqueous buffered drug solution. Key: ○, salicylic acid, 2.5 × 10⁻⁴ M and salicylic acid 2.5 × 10⁻⁴ M in presence of polysorbate 80 in concentrations of: △, 0.001%; ●, 0.005%; and ▲, 0.1%.*

(Fig. 2) to those obtained with polysorbate 20, *i.e.*, the more dilute solution of surfactant, 0.001%, is more effective in enhancing absorption. Comparing amounts of drug remaining in solution the results with 0.001% polysorbate 40 differ significantly from the control at slightly above a 90% confidence level. The average rate constant of this solution differs significantly from that of the control at a 95% confidence level. None of the other results differ significantly from the control. Complexation and surface tension measurements for this surfactant do not show any unusual effect on drug absorption.

In the case of both polysorbate 20 and 40 there appears to be a concentration above which absorption is inhibited and below which drug uptake is enhanced.

Polysorbate 60—Figure 3 shows the results with polysorbate 60. The surfactant in concentration used here seems to have less tendency toward inhibiting absorption than the two lower polysorbates. Statistical comparisons show significant differences between rate constants of the solution containing 0.001% surfactant and control while a comparison of total drug remaining in solution shows the 0.1% concentration of polysorbate 60 to differ significantly from the control. Complexation and surface tension data are similar to those of polysorbate 40.

Polysorbate 80—Results with polysorbate 80 show some similarity to those obtained with polysorbate 60. The amount of salicylic acid absorbed at the two-hour limit was significantly increased by 0.1% polysorbate 80, whereas the results with the two lower concentrations do not differ significantly from the control. The rate constants do not differ significantly from that of the control. The degree of complexation is greater with the 0.1% polysorbate concentration indicating a possible correlation to absorption.

Surface-active agents in addition to influencing drug absorption by complexation and possibly by surface tension lowering or wetting action may also affect drug uptake by an action on the absorbing membrane. This was true in goldfish as shown by Levy and Anello (6) who immersed the fish first in a surfactant solution and subsequently noted an increase in drug absorption from a drug solution without surfactant.

Pre-immersion of the frog for 15 min. in solutions of the polysorbates used in this study failed to have any effect on the subsequent absorption of salicylic acid from aqueous buffered solutions.

DISCUSSION

Complexation may play a part in the absorption of salicylic acid by the frog. For two of the surfactants used, the increase in absorption corresponded to an increase in complexation when considered from the standpoint of total drug absorbed during the experiment. Surface tension lowering may play a part by permitting better wetting action of the solution on the skin of the frog but there is no direct or readily apparent correlation.

In comparing the results with the different polysorbate it appears that more dilute concentrations of polysorbate 20 and polysorbate 40 give similar effects to the more concentrated solutions of the two higher polysorbates.

It is believed that a wider range of concentration of polysorbates might produce more apparent and significant differences in results. This would permit a consideration of the effect of critical micelle

concentration on absorption. Present research is proceeding along these lines.

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Calculations of Release Rates From Sustained-Release Dosage Forms Using the Wiley Method

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Abstract □ In the Wiley method for determining release rates from sustained-release formulations, typically, each hourly sample is assayed. If release specifications include intervals of more than 1 hr., a method is presented by which samples can be combined in a manner such that it is not necessary to assay each hourly sample. In cases where the assay is tedious or difficult, or in cases where multiple assays are necessary, this procedure may result in a substantial saving of time.

Keyphrases □ Release rates—sustained-release dosage forms □ Wiley method—release rate calculations □ Sample combination—release rates

Among the methods proposed and described in the literature for *in vitro* testing of release rates from sustained-release formulations are those which rely on hourly assay samples taken from a continuous extraction solvent (1–6). One such procedure, in current use, was originally proposed by Wiley (1). The material to be assayed is packed in a specially designed column and 100 ml. of simulated gastric fluid is circulated through the column by means of a pump. After each hour, 50 ml. of fluid is removed for assay and replaced with 50 ml. of simulated intestinal fluid. Thus, the ratio of intestinal to gastric fluid in the extracting solvent is increased with each succeeding hour. Since the calculation of total amount released during a given hourly interval is dependent on the assay of the previous hourly sample, (*vide infra*), release rates are generally calculated for each hour. This paper is concerned with a method by which release rates can be determined over intervals of more than 1 hr. without assaying each and every individual hourly sample.

METHOD AND THEORY

If the method, as described above is used, the calculation for drug released during hour $(n-1)$ to (n) is $C_n = A_n - {}^1/_2 A_{n-1}$, where A_n = assay of total active ingredient per 100 ml. of solution after hour (n).[1]

Example 1:

Hr.	C_n	A_n
1	$C_1 = 10$	$C_1 = 10 = A_1$
2	$C_2 = 31$	$C_2 + {}^1/_2 A_1 = 36 = A_2$
3	$C_3 = 12$	$C_3 + {}^1/_2 A_2 = 30 = A_3$

e.g., $C_3 = A_3 - {}^1/_2 A_2 = 30 - {}^1/_2 (36) = 12$

Suppose that one is not interested in the quantity released each and every hour, but, rather, between certain specified hours, *e.g.*, between Hours 1 and 3. The question is: is it necessary to assay the samples obtained during the intermittent hours, *e.g.*, Hour 2, in order to calculate the total amount released during, *e.g.*, Hours 2 and 3? Remember that the fluid must still be changed every hour.

If hourly samples are properly combined, it is not necessary to separately assay the samples from intermittent hours. Consider Example 1. If 25 ml. of the Hour 2 sample is combined with 50 ml. of the Hour 3 sample and assayed, the total calculated amount of active material released will be $(C_3 + {}^1/_2 A_2) + ({}^1/_2 A_2) = C_3 + C_2 + {}^1/_2 C_1$. Thus, with a knowledge of C_1, $C_3 + C_2$ may be calculated.[2]

In general, the solution to the problem is not as simple as in the above example. Fortunately, there exists a rather straightforward solution to the problem of mixing samples and the calculation of the amount released between specified hours as follows:

Indicate hours at which time release limits are specified, *e.g.*, 1, 3, 6, and 7 hr., *i.e.*, one wishes to know the amount released after 1 hr., amount released between 1 and 3 hr., between 4 and 6 hr., and 6 and 7 hr. These hours would be called (1, 3, 6, 7) assay hours for convenience.

When hourly samples are combined, the samples collected at assay hours will never be combined with each other. Save all hourly samples (50 ml. in the present case) in numerical order and combine as follows:

To each assay hour sample, first add one-half the quantity of the previous hourly samples until another assay hour sample is reached. Ignore this sample and then proceed to add one-fourth the quantity of the next prior hourly samples until another assay hour sample is reached. Then add one-eighth of the prior samples, *etc.* Note that the fraction of samples added decreases in integral powers of $({}^1/_2)$ and that if two successive assay hours are encountered, the addition of the prior samples is decreased by one-half for each assay hour encountered.

The following two examples should clarify the above procedure. H_n refers to the solutions removed for assay after hour (n).

[1] Since only 50 of 100 ml. of solution are taken for assay, the total amount of active ingredient in the sample, A_n, will be twice the amount found in the 50 ml.

[2] For this and subsequent calculations, when samples are combined, the combined sample is treated as having a volume of 50 ml. Thus, to calculate the total amount in 100 ml., the amount found in the combined sample should be multiplied by 2. For example, if 24 mg. is found in a combined 75-ml. sample, 48 mg./100 ml. is calculated.