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An analogue computer study of the dialysis of salicylic acid from micellar solutions of polysorbate 20 and 80

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The equilibrium dialysis of salicylic acid from solutions of polysorbate 20 and 80 has been investigated. The system has been simulated using an analogue computer. Rate constants for the partition of salicylic acid out of polysorbate micelles into water have been obtained by fitting computer generated curves to experimentally determined data. Under the present experimental conditions the release of salicylic acid from polysorbate micelles is independent of surfactant concentration but depends on the micelle : aqueous partition ratio of salicylic acid and its concentration in the donor and recipient cells.

In a previous report, rate constants for the dialysis of salicylic acid from polysorbate solutions were determined (Withington & Collett, 1973). The pseudo-phase model of surfactant solutions was used to describe the system and as a basis for calculating theoretical dialysis rate constants from solubilization data (Withington & Collett, 1973). Use of the pseudo-phase model of surfactant solutions implies that as solubilizate dialyses from the donor aqueous phase, solubilizate will partition out of the surfactant micelles into the donor aqueous phase in order to maintain the constancy of the micellar : aqueous partition coefficient. In calculating theoretical dialysis rates for dialysis times up to 120 min it was assumed that the amount of salicylic acid transferred from the micellar to the aqueous phase was insignificant. The validity of this assumption is substantiated by

(i) the linearity of first order plots for salicylic acid dialysis from polysorbate solutions. These plots would be expected to exhibit curvature if two consecutive kinetic processes were involved in the transfer of salicylic acid across the dialysis membrane.

(ii) the ability of equations based on the assumption to predict successfully the dialysis rate of salicylic acid in the presence of polysorbates.

When dialysis is allowed to proceed for more than 120 min the amount of salicylic acid partitioning out of the polysorbate micelles can be expected to become significant and influence the overall rate of dialysis. Different surfactants may influence the rate to different extents. A measure of the extent of this influence may be useful in understanding the effects of polysorbates on the rate of salicylic acid absorption *in vivo*. In this report the kinetics of salicylic acid transfer from the micelles of two polysorbates are investigated using the electronic analogue computer.

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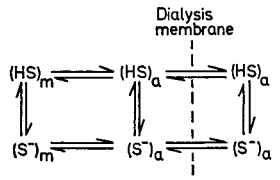

FIG. 1. A schematic representation of salicylic acid transfer from micellar surfactant solutions across a dialysis membrane (- - -). (HS) and S^-) are the unionized and ionized acid respectively and subscripts a and m denote aqueous and micellar phases respectively.

THEORY

Drug transfer from aqueous surfactant solutions across a dialysis membrane can be considered to consist of two distinct processes: (a) transfer between surfactant micelles and water, and (b) transfer from the water across the dialysis membrane. Fig. 1 represents these processes as a series of reversible reactions. For convenience, analogue computer studies are limited to the case when only unionized salicylic acid is present in solution. The part of Fig. 1 that is applicable to this case is

$$(HS)_m \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (HS)_a \quad \text{(Dialysis membrane: } k_2 \text{ / } k_{-2}\text{)} \quad \rightleftharpoons (HS)_a \qquad \ldots \quad (1)$$

where k_1 and k_2 are the clearances for unionized salicylic acid transfer from polysorbate micelles to water and from water on one side of the membrane to water on the other side. k_{-1} and k_{-2} are the corresponding clearances for the reverse processes (clearances are first order rate constants multiplied by compartment volumes). Equation (1) may be written as

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \qquad \ldots \quad (2)$$

where A is the micellar salicylic acid concentration and B and C are the aqueous salicylic acid concentrations on the donor and recipient sides of the membrane respectively. The rate of change of the amount of salicylic acid in each compartment with time is given by the equations

$$V_a \frac{dA}{dt} = -k_1 A + k_{-1} B \qquad \ldots \quad (3)$$

$$V_b \frac{dB}{dt} = k_1 A - k_{-1} B + k_2 (C - B) \qquad \ldots \quad (4)$$

and

$$V_c \frac{dC}{dt} = k_2 (B - C) \qquad \ldots \quad (5)$$

where the volumes, V, of each compartment are denoted by the appropriate subscript and in which k_2 has been substituted for k_{-2} since $k_{-2} = k_2$.

At equilibrium

$$V_a \frac{dA}{dt} = O \qquad \ldots \quad (6)$$

and therefore from equation (3)

$$k_1 A = k_{-1} B \quad .. \quad .. \quad .. \quad (7)$$

Rearranging

$$k_1/k_{-1} = A/B \quad .. \quad .. \quad .. \quad (8)$$

but A/B is the micellar: aqueous partition coefficient, P, (Collett & Withington, 1972) of unionized salicylic acid so that

$$k_1/k_{-1} = P \quad .. \quad .. \quad .. \quad (9)$$

or

$$k_{-1} = P\,k_1 \quad .. \quad .. \quad .. \quad (10)$$

Equation (3) can now be written

$$V_a \frac{dA}{dt} = -k_1 A + P\,k_1 B = k_1 (PB - A) \quad .. \quad .. \quad (11)$$

Substituting equation (10) into equation (4) and removing the volume term from the left-hand side gives

$$\frac{dB}{dt} = \frac{k_1}{V_b}(A - PB) + \frac{k_2}{V_b}(C - B) \quad .. \quad .. \quad .. \quad (12)$$

or, changing signs

$$\frac{dB}{dt} = -\frac{k_1}{V_b}(PB - A) - \frac{k_2}{V_b}(B - C) \quad .. \quad .. \quad .. \quad (13)$$

Removing V_c from the left-hand side of equation (5) gives

$$\frac{dC}{dt} = \frac{k_2}{V_c}(B - C) \quad .. \quad .. \quad .. \quad (14)$$

On introducing V_c into equation (13) part of the right-hand side of that equation becomes identical to the right-hand side of equation (14):

$$\frac{dB}{dt} = -\frac{k_1}{V_b}(PB - A) - \frac{V_c}{V_b}\frac{k_2}{V_c}(B - C) \quad .. \quad .. \quad .. \quad (15)$$

Introducing voltage scaling factors (fA), (fB), and (fC), and a time scaling factor (fT), equations (11), (14) and (15) can be written

$$\frac{d(VA)}{dt} = \frac{(fT)\,k_1}{V_a}\left[P\frac{(fB)\,(VB)}{(fA)} - (VA)\right] \quad .. \quad .. \quad .. \quad (16)$$

$$\frac{d(VB)}{dt} = -(fT)\frac{k_1}{V_b}\frac{(fA)}{(fB)}\left[P\frac{(fB)\,(VB)}{(fA)} - (VA)\right] \quad .. \quad .. \quad (17)$$

$$-\frac{V_c}{V_b}\frac{k_2}{V_c}(fT)\frac{(fC)}{(fB)}\left[\frac{(fB)\,(VB)}{(fC)} - (VC)\right]$$

and

$$\frac{d(VC)}{dt} = (fT)\frac{k_2}{V_c}\left[\frac{(fB)\,(VB)}{(fC)} - (VC)\right] \quad .. \quad .. \quad .. \quad (18)$$

The analogue computer program fitting these three equations is shown in Fig. 2.

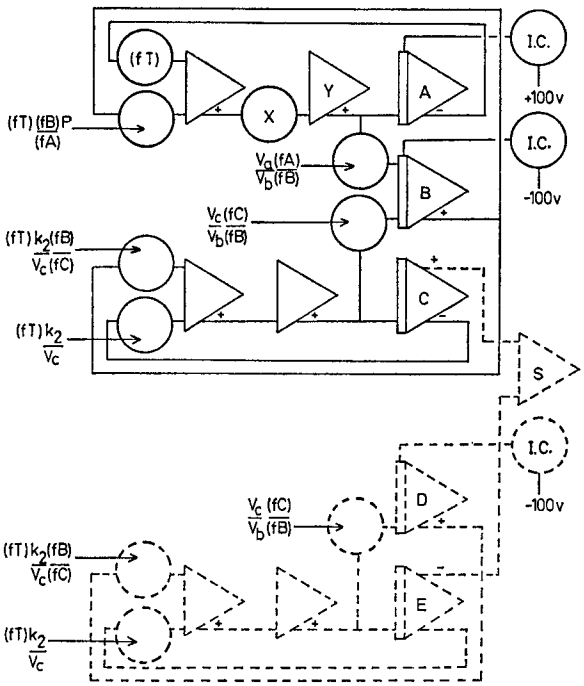

FIG. 2. Analogue computer program for simulation of dialysis from surfactant solutions. Integrators A, B and C represent the three compartments of the model (equation 2). Quantities written inside potentiometers are the quantities appearing in equations (16), (17) and (18). The product of x and y represents the clearance k_1. The broken line is the program for simulation of dialysis from salicylic acid solutions containing no surfactant. Amplifier S computes the difference between the outputs of integrators C and E.

METHODS

The analogue computer provides simulations of the complete dialysis process up to equilibrium. Corresponding data must be obtained from the real dialysis system so that comparisons may be made over the whole period of dialysis.

The experimental procedure for studying the dialysis of salicylic acid from polysorbate solutions has already been described (Withington & Collett, 1973). However, the method has been modified because samples must be taken frequently over periods greater than 25 h and using the previous sampling technique would involve removal of a large volume of the dialysing solutions during the course of dialysis. A continuous sampling technique was introduced in which dialysis solution was recirculated through a flow cell situated in a spectrophotometer.

Experimental procedure for studying dialysis to equilibrium

The dialysis cell was assembled and placed in its water bath as described previously (Withington & Collett, 1973). 100 ml of solvent (0·1M hydrochloric acid) was placed in the recipient half cell and the stirrer motors started. Recipient solution was pumped by a peristaltic pump (Watson-Marlow H.R. Flow Inducer, type HRE 200) through polythene tubing (1·0 mm i.d.) into a 1·0 mm path length quartz spectrophotometer cell (Chandos Intercontinental) and returned to the recipient half cell. The flow cell was held in the beam (298 nm) of a spectrophotometer (Pye Unicam

SP 500). Flow rate through the flow cell was 1·6 ml min^{-1} and the volume of solution in the tubing and flow cell at any one time was less than 2 ml. After setting the absorbance reading of the spectrophotometer to zero, 100 ml of solution of salicylic acid in 0·1M hydrochloric acid was added to the donor half cell and a chart recorder (Smiths Industries servoscribe), monitoring the output of the spectrophotometer, was started (chart speed: 30 mm h^{-1}).

Preliminary experiments indicated that during the time of the experiment the spectrophotometer output showed appreciable drift which was presumably due to ageing of the deuterium lamp. Effects of this drift on the experimental results can be minimized by observing the output of the spectrophotometer corresponding to transmissions of 0 and 100% as a function of time. In order to observe these outputs during the course of dialysis experiments an automatic cell changer (Pye Unicam S 505), controlled by a time switch (Pye Unicam SP 505), was used in place of the standard, manual, cell changer. Three compartments of the cell changer contained the following: (a) the flow cell through which recipient solution was recirculated, (b) a black card which prevented ultraviolet radiation reaching the photocell. When this compartment was in the light beam the spectrophotometer output corresponded to 0% transmission, (c) a black card which only partially obscured the light beam and whose position could be adjusted to give a spectrophotometer output equivalent to 100% transmission.

A sequence of changes was programmed on the time switch so that every 15 min cell compartments b and c, corresponding to 0 and 100% transmission respectively, were each placed in the light beam. The cells remained in the light beam for about 8 s and the spectrophotometer output was automatically recorded on the recorder. During each 15 min period compartment a, containing the flow cell, was in the light beam for all but the 30 s interval required for the cell change sequence and a virtually continuous record of transmission through the recipient solution was obtained.

Examination of the recorder traces obtained using the above method showed that the spectrophotometer output corresponding to 0% transmission decreased by up to 4·5% over 48 h. At any given time, transmission through the recipient solution was obtained by expressing the distance between 0% transmission and the line recording solution transmission as a percentage of the distance between 0 and 100% transmission on the recorder trace.

Analogue computer simulation of dialysis to equilibrium

An analogue computer (Applied Dynamics, A.D.256) was programmed according to Fig. 2. Settings on the initial condition potentiometers were calculated, using the appropriate voltage scaling factors, from the initial concentrations* of salicylic acid in the micellar and aqueous phases of the polysorbate solutions used in the dialysis experiments. The remaining potentiometers were set at values calculated according to Equations (16), (17) and (18) (see also Fig. 2). An X-Y recorder was used to monitor the output of integrator C as a function of time.

RESULTS AND DISCUSSION

Computer generated curves fitting the experimental dialysis results were obtained by optimizing the settings of X and Y (Fig. 2), which correspond to K_1, the clearance

* Micellar and aqueous phase concentrations of salicylic aid were calculated using previously reported (Collett & Withington, 1973) equations.

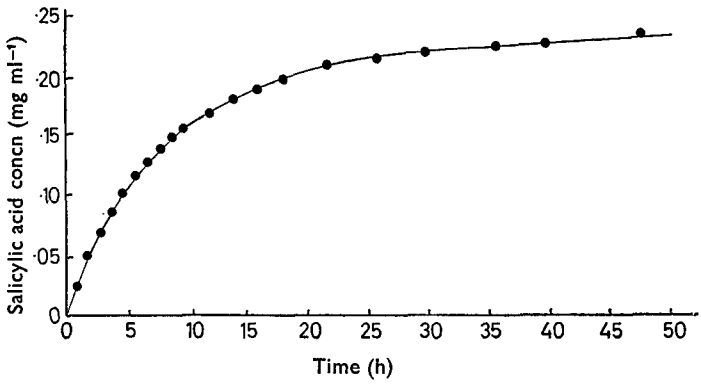

FIG. 3. The dialysis of salicylic acid from 2·0% w/v polysorbate 20 solutions. The points are experimentally determined recipient cell concentrations and the line is the analogue computer fitted curve.

rate for partition of salicylic acid out of polysorbate micelles. Typical results are shown in Fig. 3.

Relatively large changes in computer variables representing k_1 produced relatively small changes in the computer drawn dialysis curves indicating that the precision of k_1 values calculated from this program was probably quite low. Because the magnitude of k_1 is primarily responsible for the difference between dialysis curves obtained in the absence and presence of polysorbate, a computer method based on this fact may be capable of providing relatively precise estimates of k_1.

Using the following treatment, the difference in concentration at any time between dialysis curves obtained in the absence and presence of polysorbate has been calculated from experimental data. Non-linear regression analyses of dialysis data showed that the dialysis curves were fitted (correlation coefficient greater than 0·9987) by a quartic equation. When dialysis was from water the concentration, C_a, of salicylic acid in the recipient cell at any time, t, is given by the equation

$$C_a = q_0 + q_1 t + q_2 t^2 + q_3 t^3 + q_4 t^4 \quad .. \quad .. \quad .. \quad (19)$$

where q_0, q_1 . . . . are the appropriate regression coefficients.
When dialysis is from polysorbate solution the salicylic acid concentration, C_r, in the recipient cell is given by

$$C_r = q_5 + q_6 t + q_7 t^2 + q_8 t^3 + q_9 t^4 \quad .. \quad .. \quad .. \quad (20)$$

At any time the difference in salicylic acid concentration between the two dialysis systems is given by

$$C_a - C_r = (q_0 - q_5) + (q_1 - q_6)t + (q_2 - q_7)t^2 + (q_3 - q_8)t^3 + (q_4 - q_9)t^4 \quad (21)$$

Fig. 4 shows dialysis curves for salicylic acid from water and from a polysorbate solution. Salicylic acid concentration in the recipient compartment at equilibrium was arranged to be the same in each case. The difference in concentration at any time between dialysis in the absence and presence of polysorbate was calculated, using equation (21), from the quartic regression coefficient of each curve, and is also plotted in Fig. 4. The position and peak height of the concentration curve is determined by the magnitude of k_1 and may be used as a basis for comparison with similar, computer generated curves.

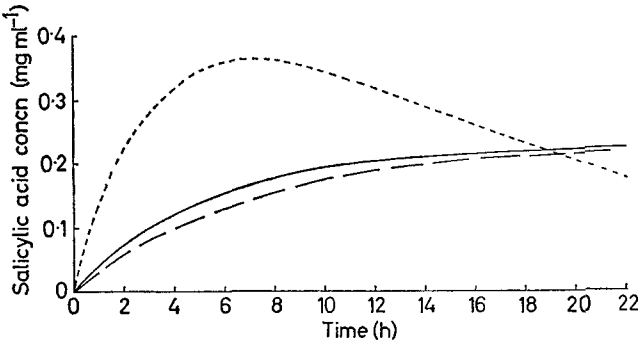

FIG. 4. A concentration difference curve for the dialysis of salicylic acid. Recipient cell concentrations of salicylic acid, when dialysis is from polysorbate solutions (———), are subtracted from those obtained when dialysis is from water (— —). The difference is multiplied by 10 and plotted (- - - - -) as a function of time.

The computer program was modified so as to give concentration difference curves and is shown in Fig. 2. That part of the program drawn in solid lines is identical to the program used previously and the part drawn in broken lines represents the dialysis of salicylic acid from water. These two parts of the program simultaneously generate curves for dialysis from polysorbate solutions and from water, respectively, and the difference between their output is computed at amplifier S. By systematically varying the settings of computer elements representing k_1 the recorded output of amplifier S can be adjusted to coincide with experimentally determined concentration difference curves. Fig. 5 shows typical results of this type of study.

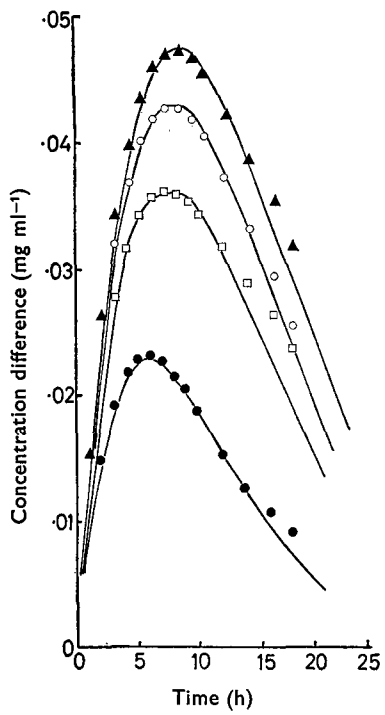

FIG. 5. The difference between recipient cell concentrations of salicylic acid dialysed from water and from solutions containing ● 1·0; □ 2·0; ○, 4·0; and ▲, 5·0% w/v polysorbate 20. The points were determined from experimental data and the lines are the analogue computer fitted curves.

Table 1. *Analogue computer determined values of micellar : aqueous clearances, k_1, of salicylic acid in polysorbate 20 and 80 solutions.*

Surfactant	Surfactant concentration (% w/v)	Initial salicylic acid concentration[d] (mg ml^{-1})	k_1 (ml min^{-1}) $\times 10^3$ Program A[a]	Program B[b]
Polysorbate 20	1·14	0·7	0·68	0·68[c]
,,	2·28	0·9	0·41	0·37
,,	4·58	1·35	0·54	0·37
,,	5·71	1·50	0·44	0·38
Polysorbate 80	1·11	0·75	2·09	1·17
,,	2·23	1·0	4·68	2·94[c]
,,	4·46	1·6	1·34	1·34[b]
,,	5·58	1·8	1·33	1·34

[a] Program A generated from straightforward dialysis curves.
[b] Program B generated from concentration difference curves.
[c] The fit between computer curve and experimental data was very poor.
[d] Calculated using equation (20) (Withington & Collett, 1973b) such that at equilibrium the concentration of salicylic acid in the recipient cell was approximately 0·25 mg ml^{-1}.

Although computer generated curves do not appear to fit experimental data as closely as was the case for straightforward dialysis, estimates of k_1 derived from the results are more reliable. Small changes in computer variable representing k_1 produced relatively large changes in the shape of the computer drawn curves enabling k_1 to be determined more accurately than with the previous program.

Values of k_1 were calculated from the settings of X and Y (Fig. 2) required to provide a good fit between computer generated and experimentally determined curves. Values of k_1 obtained using each computer program are presented in Table 1. For polysorbate 20, k_1 is constant with the exception of the value at 1·0% w/v surfactant. A similar constancy is observed in the case of polysorbate 80 except for 2% w/v surfactant. This constancy suggests that the rate, as opposed to rate constant, of release of salicylic acid from polysorbate micelles is independent of surfactant concentration and depends only on the value of (PB — A). For polysorbate 80, k_1 is approximately 4 times greater than for polysorbate 20 which means that, under the present experimental conditions for a given value of (PB — A), (equation 22), salicylic acid is released from polysorbate 80 micelles four times faster than from polysorbate 20 micelles. This difference is probably accounted for by structural differences in the micelles which allow salicylic acid to leave micelles of polysorbate 80 more rapidly than those of polysorbate 20.

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