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The transfer of salicylic acid across a Cellophane membrane from micellar solutions of polysorbates 20 and 80

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In the pH range 1 to 5 there is a linear relation between the rate constant for salicylic acid transfer across a cellophane membrane and the fraction of ionized drug present at each pH. The rate constant for ionized molecules is about 60% of that for unionized molecules. The presence of polysorbates 20 and 80 in the drug solution markedly decreases the apparent transfer rate constant of salicylic acid at low pH. When transfer rate constants obtained in the presence of surfactant are calculated in terms of the non-micellar concentration of salicylic acid they compare favourably with those obtained in the absence of surfactant. These results indicate that micellar drug does not participate in the transfer process.

The marked reduction of drug absorption that is frequently observed in the presence of surfactants is usually attributed to solubilization (Gibaldi & Feldman, 1970) which is thought to reduce the amount of drug that is available for absorption (Allawala & Riegelman, 1953; Humphreys, Richardson & Rhodes, 1968). However, because of the complexity of biological systems quantitative physico-chemical interpretations of surfactant effects on *in vivo* drug absorption are not forthcoming; therefore an understanding at the *in vitro* level must first be obtained.

Previously (Collett & Withington, 1972) we have reported drug partition coefficients for ionized and unionized salicylic acid between water and a series of non-ionic surfactants differing only in their hydrophobe. Using these partition coefficients the amount of drug in the aqueous and micellar phases of solutions of each surfactant can be calculated. The importance of such calculations is that they may be useful for estimating the amount of the drug available for transfer in an *in vivo* system. However it is too great a step from the *in vitro* determination of partition coefficients to their application to an *in vivo* system without first investigating rates of drug release *in vitro*. In this paper the effect of two surfactants on the transfer of salicylic acid across a cellophane membrane is reported. The rate of transfer in the presence of the surfactants has been correlated with the previously determined micellar: aqueous partition coefficients for ionized and unionized salicylic acid molecules.

MATERIALS AND METHODS

Materials

The two surfactants used have been described by Collett & Withington (1972). Salicylic acid was analytical reagent grade (BDH). Cellophane (Visking, supplied by the Scientific Instrument Centre Ltd.) was boiled for 10 min in each of three changes of distilled water and stored in distilled water until required for use. The thickness of the wet membrane was 0·005 cm.

The dialysis cell

A diagram of one of the Perspex half cells is shown in Fig. 1. The capacity of each half cell is 150 cm^3 and when both contain 100 cm^3 of solution 18·9 cm^2 of membrane surface is in contact with each solution. The three bladed stirrers are attached to 300 rev min^{-1} motors (Crouzet) mounted directly above each half cell. In the collar through which the shaft of each stirrer passes provision is made for the insertion of pH electrodes and a titrant delivery tube. A fourth hole in the collar serves as a sampling port. Temperature control was achieved by immersing the dialysis cell assembly in a water bath (25 ± 0·01°) to the level of the dialysing solutions.

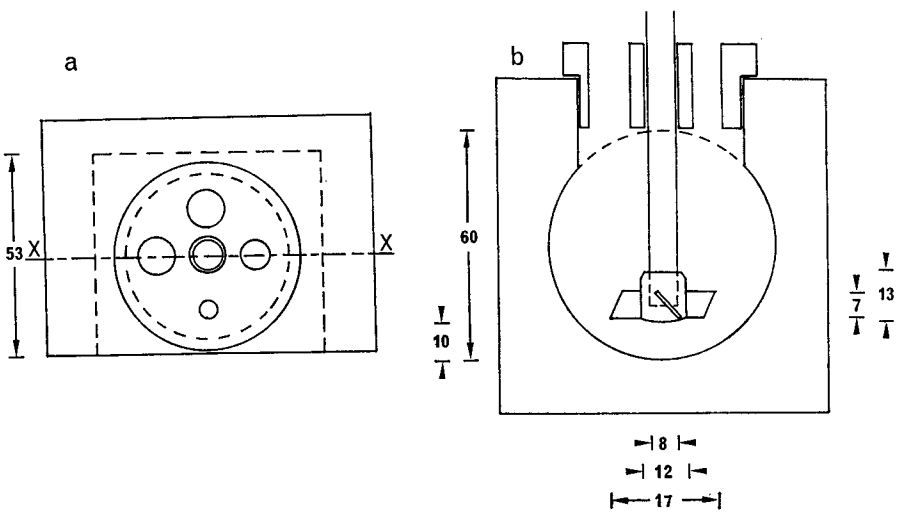

FIG. 1. Diagram of one half cell. (*a*) Top view with the collar through which the stirrer shaft, pH electrodes and titrant delivery tube pass and (*b*) a section through X – X. Only the hydrodynamically significant dimensions are given (mm).

Procedure

A piece of Cellophane membrane was clamped between the two half cells and the complete cell placed on a platform in the water bath. 100 cm^3 of salicylic acid solution (1·0 g $litre^{-1}$) was placed in the donor half cell and the stirrer motors started. The pH of the solution was adjusted to the pH required with HCl or NaOH solutions. At time zero 100 cm^3 of distilled water at 25° adjusted to the same pH as the donor solution was added to the recipient half cell. During dialysis the pH of each solution was monitored and maintained by the addition of HCl or NaOH solutions under the control of two pH stats (Radiometer, Copenhagen).

At suitable time intervals 1·0 cm^3 samples were removed from the recipient half cell, diluted appropriately with 0·1 M HCl and assayed spectrophotometrically (Pye-Unicam SP 500 instrument) for salicylic acid at 298 nm. Dialysis was continued for 90 to 120 min.

RESULTS AND DISCUSSION

Kinetic analysis

The form of Fick's law applicable to the transfer of solute across a membrane is:

$$\frac{dm}{dt} = \frac{D' A(C_d - C_r)}{X} \quad .. \quad .. \quad .. \quad (1)$$

where dm/dt is the amount of solute transferred in unit time, A the area across which transfer takes place, C_d and C_r the concentrations of solute in the donor and recipient compartments respectively, X the thickness of the membrane and D′ the dialysis coefficient (Kostenbauder, Boxenbaum & Deluca, 1969). For a porous membrane D′ is a function of the solute diffusion coefficient, D, and the tortuosity and cross sectional area of the membrane pores.

When C_d and C_r vary with time and the volumes Vd and Vr of the two compartments are equal equation (1) integrates to:

$$\text{Log}\,(C_d - C_r) = -\frac{2D'At}{2{\cdot}3\ VX} + \text{constant} \quad .. \quad .. \quad (2)$$

For a particular apparatus and procedure D′, A, V and X are constant so that:

$$\text{Log}\,(C_d - C_r) = -Kt + \text{constant} \quad .. \quad .. \quad (3)$$

a plot of log $(C_d - C_r)$ *vs* t should give a straight line whose slope, K, is a first order rate constant of dialysis. In this report rates of dialysis are compared in terms of their rate constants.

Reproducibility of results

Replicate (14) experiments were carried out with one piece of membrane at pH 1·0. The mean value of K was found to be $1{\cdot}35 \times 10^{-3}$ min^{-1}. The relative standard deviation of these results was 3·47%. Subsequently pieces of membrane were re-used up to 75 times without loss of reproducibility.

Effect of experimental conditions on rate of dialysis

1. pH of solution. Experiments were carried out at pH values in the range pH 1·0 to pH 12·0. Typical results are shown in Fig. 2. The sigmoidal shape of this curve

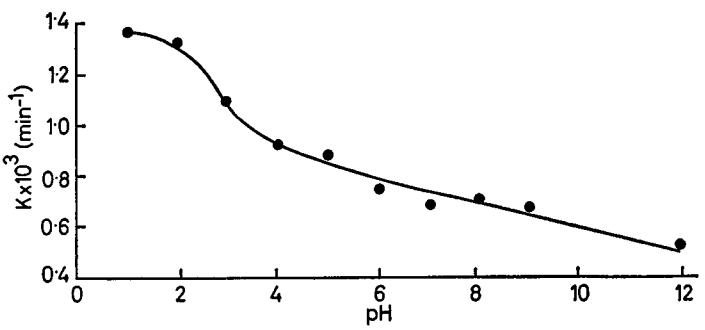

FIG. 2. The relation between the dialysis rate constant, K, and pH for salicylic acid in water.

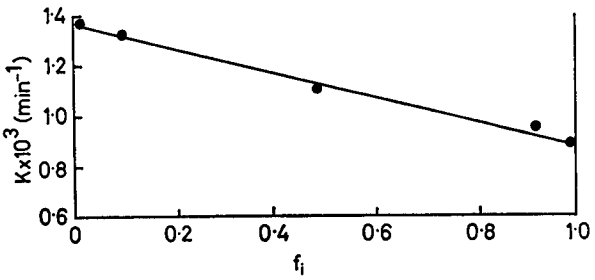

FIG. 3. Plot of dialysis rate constant, K, against the fraction, f_i, of salicylic acid ionized up to pH 5·0.

is similar to that of the fraction ionized *vs* pH curve for salicylic acid. In Fig. 3 the data of Fig. 2 are plotted as a function of the fraction, f_i, of ionized salicylic acid at each pH. The linearity of this plot in the range corresponding to pH 1·0 to pH 5·0 enables rate constants K° and K′ for unionized and ionized molecules respectively to be obtained by extrapolation to $f_i = 0$ and $f_i = 1{\cdot}0$.

Data obtained from all the experiments reported in this paper have been plotted as shown in Fig. 3 and give $K° = 1{\cdot}37 \times 10^{-3}$ min^{-1} ± 3·7% ($P = 0{\cdot}05$) and $K' = 0{\cdot}87 \times 10^{-3}$ min^{-1} ± 5·9% ($P = 0{\cdot}05$). The lower rate constant for ionized salicylic acid molecules can probably be explained in terms of their greater degree of interaction with the solvent. This would give a molecular diffusion coefficient lower than that for unionized molecules which would be reflected by a decrease in K (eqns 2 and 3).

At pH values above pH 5·0 the dialysis rate constant of ionized molecules is reduced even further. It is not clear whether this observation results from pH effects on the solute-solvent interaction or on the membrane. The hypothesis that at pH values greater than 5·0 the porosity of the membrane may be modified was tested by investigating the effect of pH on the dialysis of *p*-cresol which is almost completely unionized up to pH 8·0 (pKa = 10·8). The dialysis rate constant for this substance in the pH range 1·0 to 8·0 (Fig. 4) is $1{\cdot}47 \times 10^{-3}$ min^{-1}, relative standard deviation 2·3%. Above pH 8·0 ionization of the molecule begins and the dialysis rate constant falls in accordance with the explanation given above. That the rate constant for *p*-cresol is invariant up to pH 8·0 does not support the hypothesis that the pH effect observed with salicylic acid above pH 5·0 is the result of changes in membrane porosity.

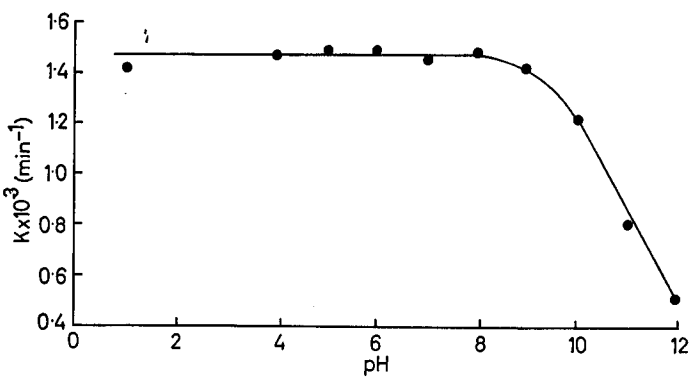

FIG. 4. The relation between the dialysis rate constant, K, and pH for *p*-cresol.

2. *Sub-micellar surfactant concentrations.* Alexander & Trim (1946) reported that surfactants may influence drug absorption at concentrations lower than their cmc. To test for the presence of surfactant effects below the cmc in the dialysis model salicylic acid was dialysed at pH 1·0 from a solution containing 0·005% w/v of polysorbate 20 (cmc of polysorbate 20 is 0·006% w/v; Mittal, 1972), into water at pH 1·0. The dialysis rate constant in this experiment was $1{\cdot}39 \times 10^{-3}$ min^{-1} which is within the limits of experimental error of the value obtained in the absence of surfactant. It is therefore concluded that at sub-micellar concentrations the surfactant does not influence the transfer of salicylic acid in this system.

3. *Viscosity of solutions.* If the rate of salicylic acid transfer from the donor to the recipient compartment is limited by diffusion of salicylic acid molecules through the bulk of the solution then the overall transfer rate will be influenced by the viscosity of the solutions. The maximum concentration of surfactants used in this study was 5% w/v and a 1·0 g $litre^{-1}$ salicylic acid solution containing this concentration of polysorbate 20 has a relative viscosity of 1·3. The effect of viscosity on the dialysis rate constant was investigated by dialysing salicylic acid from a solution whose relative viscosity was adjusted to 1·3 with 0·15% w/v methylcellulose. At pH 1·0 and 12·0 the dialysis rate constants were within the limits of experimental error of the values obtained in the absence of surfactant. Evidently the transfer process is rate limited by the membrane transfer step and not by diffusion through the bulk solution.

4. *Osmotic pressure of solutions.* When a solution is separated from pure solvent by a barrier which is permeable only to solvent molecules an osmotic pressure is established which results in a flux of pure solvent into the solution. This situation exists in the system under study when surfactant molecules are present on only one side of the membrane. Flux of water from the recipient cell to the donor cell, in the direction of the osmotic gradient, may lead to flux of salicylic acid in the same direction and against the prevailing salicylic acid concentration gradient. The osmotic pressure gradient can be removed by setting the surfactant concentration in the recipient cell equal to its concentration in the donor.

When the donor and recipient cells each contain 5% w/v polysorbate 20 the dialysis rate constant is within the limits of experimental error of the value obtained when only the donor contains this concentration of surfactant. Clearly, osmotic pressure effects are negligible in this system.

Effect of different surfactant concentrations on K

The relation between apparent dialysis rate constant, K_{app}, and polysorbate 20 concentration at three pH levels is shown in Fig. 5. Composite results are presented in Tables 1a and b. As surfactant concentration is increased dialysis rate constant is decreased. At about pH 5·0–6·0 the presence of surfactant has only a slight effect on the dialysis rate constant.

Previously, Collett & Withington (1972) found that the micellar: aqueous partition coefficients of unionized salicylic acid molecules were 75 (polysorbate 20) and 100 (polysorbate 80). Ionized salicylic acid did not partition into the micelles of either surfactant. At pH 1·0 salicylic acid is almost completely unionized in solution and in the presence of polysorbate will partition between the polysorbate micelles and water according to the partition law. The large extent of solubilization obtained at low pH is reflected in the large reductions in dialysis rate constant observed in the present

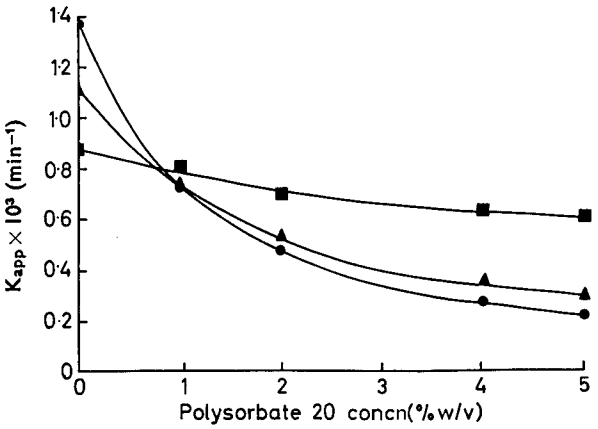

FIG. 5. Plot of the apparent dialysis rate constant, K_{app}, against polysorbate 20 concentration at various pH values ●, pH 1·0; ▲, pH 3·0; ■, pH 5·0.

study (Fig. 5, Table 1). At pH values greater than about 5·0 solubilization is minimal since nearly all the salicylic acid is ionized and cannot therefore partition into the polysorbate micelles. Thus the presence of polysorbate has little effect on the dialysis rate constant of salicylic acid at pH values greater than 5.

The driving force for dialysis is the concentration gradient of the dialysing molecules that exists across the dialysis membrane. If the concentration gradient is reduced, the rate of dialysis is also reduced although the first order rate constant will not change. The Cellophane membrane used is impermeable to polysorbate micelles (Matsumoto, Matsumura & Iguchi, 1966) so that salicylic acid solubilized within the surfactant micelles is unable to cross the membrane in micellar form. Only salicylic acid present in the aqueous phase of polysorbate solutions is therefore available for dialysis.

Table 1. *Dialysis rate constants, K, for salicylic acid in water and apparent dialysis rate constants,* K_{app}, *in polysorbate solutions at different pH values.*

(*a*) Polysorbate 20					
	$K \times 10^3$(min^{-1}) No surfactant	$K_{app} \times 10^3$(min^{-1}) Polysorbate concentration % w/v			
pH		1	2	4	5
1	1·37	0·72	0·48	0·29	0·22
2	1·33	0·72	0·47	0·28	0·22
3	1·10	0·74	0·52	0·35	0·29
4	0·92	0·82	0·71	0·55	0·51
5	0·87	0·80	0·70	0·64	0·60
6	0·74	0·67	0·68	0·62	0·62
7	0·68	0·69	0·67	0·65	0·64
8	0·70	0·67	0·65	0·61	0·63
9	0·67	0·69	0·63	0·59	0·67
12	0·52	0·52	0·52	0·50	0·47
(*b*) Polysorbate 80					
1	1·37	0·67	0·40	0·22	—
2	1·33	0·67	0·43	0·24	—
3	1·10	0·65	0·46	0·28	0·24
4	0·92	0·79	0·70	0·58	0·50
5	0·87	0·84	0·81	0·78	0·69
7	0·68	0·75	0·71	0·71	0·71

Table 2. *Dialysis rate constants,* K_{aq}, *for salicylic acid transfer from the aqueous phase of polysorbate solutions.*

(*a*) Polysorbate 20	$K_{aq} \times 10^3(min^{-1})$ Polysorbate concentration % w/v			
pH	1	2	4	5
1	1·45	1·45	1·44	1·32
2	1·40	1·35	1·29	1·23
3	1·11	1·05	1·00	0·98
4	0·88	0·80	0·69	0·68
5	0·80	0·70	0·63	0·59
6	0·66	0·66	0·59	0·59
7	0·68	0·66	0·61	0·60
8	0·67	0·63	0·58	0·59
9	0·68	0·61	0·60	0·55
12	0·52	0·51	0·48	0·44
(*b*) Polysorbate 80				
1	1·41	1·52	1·40	1·34
2	1·37	1·45	1·40	1·35
3	1·06	1·04	0·99	0·99
4	0·86	0·82	0·80	0·72
5	0·84	0·81	0·78	0·69
7	0·75	0·70	0·68	0·67

The effect of the polysorbates is to reduce the concentration gradient of transferable molecules although the total drug concentration is unchanged.

The aqueous concentration of salicylic acid in polysorbate 20 and 80 solutions can be calculated from the micellar: aqueous partition coefficients of salicylic acid, surfactant concentration, total salicylic acid concentration and the fraction of unionized salicylic acid present at the pH of the solution. By subtracting the concentration of salicylic acid in the recipient cell at each time interval the aqueous concentration gradient is obtained. The slope of a first order plot of this value *vs* time gives K_{aq}, the rate constant for dialysis between the aqueous phase of the surfactant solution and the recipient solution. Rate constants calculated in this manner (Table 2) may be compared with those obtained in the absence of surfactant (Table 1).

At low pH values there is good agreement between K_{aq} and K indicating that the concept of surfactant solutions as two-phase systems (Stainsby & Alexander, 1950) and the availability of drug molecules from the aqueous phase only (Allawala & Riegelman, 1953; Humphreys, Richardson & Rhodes, 1968) is a valid one. At higher pH levels there is some divergence between K_{aq} and K which may be indicative of an interaction between the polysorbate and ionized salicylic acid molecules. Such an interaction was not evident in our previous study.

It is clear that the effect of solubilization by polysorbates 20 and 80 on salicylic acid transfer in *in vitro* systems is severe.

The possibility of correlating the results presented here with data obtained in *in vivo* situations is at present being investigated.

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