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Permeation of Drug through a Model Membrane consisting of Millipore Filter with Oil[1]

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An investigation was done on the permeation of drug through the model membrane consisting of Millipore filter saturated with an oil, indicating that the method employed here might be satisfied for obtaining the permeation curve with an excellent reproducibility. The theoretical treatment of the data obtained by this method proved that the permeation of drug through the present system depended on the diffusion mechanism.

The ionized molecules of salicylic acid and aminopyrine did not permeate through the membrane. The temperature dependence of the permeation was well expressed by Stokes-Einstein's equation concerning diffusion constant. The experiment using different oils showed that the permeability constant increased with the increase in distribution constant in oil and decreased with the increase in viscosity of oil.

Actually, the apparent permeability constant increased proportionally with the increase in distribution constant in oil, and decreased with the increase in the viscosity of the oils used.

Keywords——model membrane; Millipore filter; oils; drug permeation; diffusion mechanism; pH dependence; temperature dependence

Permeation of drugs through various kinds of partition membranes such as silicone,[3] and dialysis membrane such as cellophane,[4] or collagen[5] has been widely investigated as an approach to an understanding of biopharmaceutical phenomena.

Upon carrying out the drug permeation experiment using these kinds of membranes with a dialysis or diffusion cell, there often arises a difficulty in obtaining the well reproducible data, as may be due to variation in properties of the membrane prepared. Additionally, another difficulty usually remains in obtaining an intrinsic diffusion constant in the membrane itself.

In the present study, the permeation of salicylic acid and aminopyrine through the membrane consisting of Millipore filter with oil was investigated with a view of finding a new model membrane suitable to a drug permeation experiment probably free from the above two difficulties. The diffusion mechanism is also discussed.

Experimental

Material——Salicylic acid and aminopyrine used were of reagent grade. The oils used to saturate the Millipore filter were also of reagent grade.

Apparatus——The glass diffusion cell devised for this study is shown in Fig. 1. The cell, which was assembled of three parts attached tightly with the binding accessories at the positions of G_1 and G_2, was

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4) *a*) N. Nambu, T. Nagai, and H. Nogami, *Chem. Pharm. Bull.* (Tokyo), **19**, 808 (1971); *b*) N. Nambu, S. Sakurai, and T. Nagai, *ibid.*, **23**, 1404 (1975).

5) M. Nakano, A. Kuchiki, and T. Arita, *Chem. Pharm. Bull.* (Tokyo), **24**, 2345 (1976).

kept in the constant temperature bath controlled within a deviation of 0.1° using a Komatsu-Yamato Coolnics CTE-220 circulator.

The membrane was set at the position of M_1 using two silicone rings in the same way as described in a previous paper.[6] Each compartment contained 150 ml of either donor or receptor solution, which was stirred well with the magnetic stirrers S located at the bottom of the compartment. The speed of the stirrers was controlled by adjusting the speed of the respective rotating magnets located under the bath, so that the stirring condition in both compartments was the same. M_2 shows the position of another membrane which may be used for a study of drug release from the ointment base, as was partly reported.[1]

Membrane——Millipore filter No. VSPO 4700 of pore diameter 25 mμ±3 mμ was soaked for 24 hr in each kind of oil in a petli dish. The amount of oil held in the Millipore membrane was about 10 mg/cm², which was constant in any case.

Procedure——This was according to the usual dialysis method. The concentration of salicylic acid and aminopyrine was determined at 302 and 264 nm respectively according to the UV absorption method, using a Beckman DB-GT spectrophotometer.

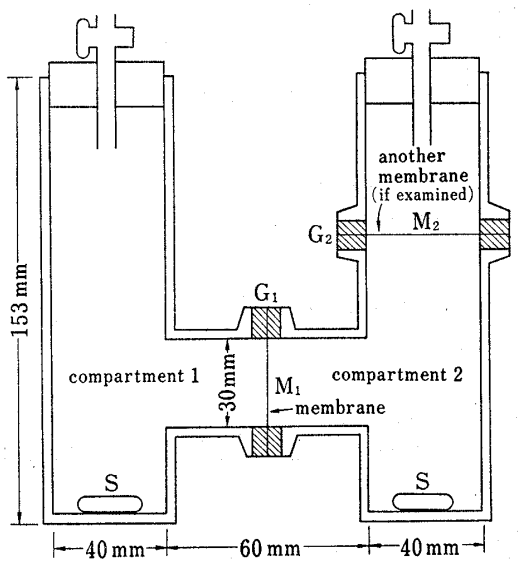

Fig. 1. The Diffusion Cell Apparatus

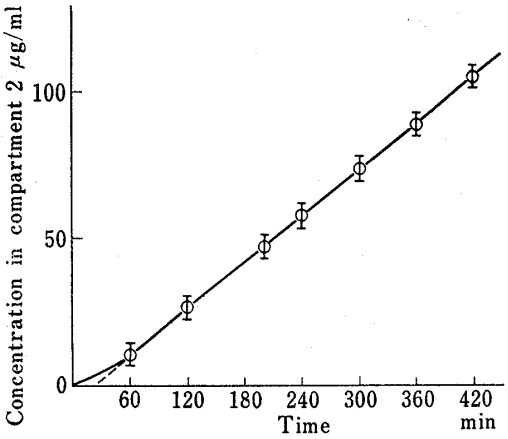

Fig. 2. A Straight Line Relationship between the Concentration of Salicylic Acid in the Compartment 2 and the Lapse of Time

⊕ shows the 95% confidence limit.

Results and Discussion

Reproducibility of Data with the Diffusion Cell

Reproducibility of data with the diffusion cell was checked in 1000 μg/ml salicylic acid in donor solution through the olive oil saturated Millipore filter membrane at 35°. The concentration of salicylic acid in the receptor solution was determined at appropriate time intervals. The result is shown in Fig. 2. A straight line relationship was obtained between the concentration of salicylic acid in the receptor solution with the lapse of time.

The lag time was estimated as 30 min by extrapolating the straight line to zero concentration of salicylic acid in the receptor cell. As shown in Fig. 2, the range of 95% confidence limit was narrow. Therefore, the reproducibility of data with the present diffusion apparatus was concluded remarkably fine.

Permeation Rate of Salicylic Acid

The theoretical equations of permeation through a diffusion cell are derived as follows:[7]

6) H. Nogami, T. Nagai, and T. Sonobe, *Chem. Pharm. Bull.* (Tokyo), **18**, 2101 (1970).
7) L.M. Lueck, D.E. Wurster, T. Higuchi, A.P. Lemberger, and L.W. Busse, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 694 (1957).

$$\log[(C_0-2C)/C_0] = [(-0.869)A \cdot D^*/l \cdot V]t \quad (1)$$

$$P^* = (-0.869)A \cdot D^*/l \cdot V \quad (2)$$

$$D^* = K \cdot f \cdot D \quad (3)$$

where C_0 is the initial concentration in the compartment 1 from which the drug permeates to the compartment 2, C the concentration in the compartment 2 at time t, A the apparent surface area of the membrane, V the volume of solution in the respective compartments, l the thickness of the membrane, D^* the apparent diffusion constant, P^* the apparent permeability constant, K the distribution constant, f the membrane constant expressed as the tortuosity divided by fraction area, and D the diffusion constant.

In the present system, the value of f was assumed to be 1.33 where tortuosity and fraction area were assumed to be 1 and 0.75, respectively.[8]

According to equations (1), (2) and (3), the permeation rate of salicylic acid through olive oil saturated Millipore filter membrane, the values of P^* and D were obtained in various initial concentrations of the donor solution, as shown in Table I.

Table I. P^* and D obtained in Various Initial Concentration of the Donor Solution

Initial concentration (μg/ml)	Lag time, t_0 (min)	Apparent permeability constant, P^* (min^{-1}, $\times 10^4$)	Diffusion constant, D ($cm^2/min \times 10^5$)
1400	18	2.82	2.89
1200	18	3.00	3.07
1000	18	3.00	3.07
700	18	3.00	3.07

P^* was almost constant in the range between 700 to 1400 μg/ml of the initial concentration of donor solution. Therefore, the permeation was considered to be of the first order.

pH Dependence of Drug Permeation

pH dependence of the permeation of salicylic acid and aminopyrine through the olive oil saturated Millipore filter was investigated in various pH of the solutions. The result is shown in Table II. P^* decreased with increase in the ionization. At the pH where most of the drug was considered to be ionized, no permeation took place in both cases of salicylic acid and aminopyrine.

Table II. pH Dependence of Salicylic Acid and Aminopyrine Permeation

pH	% ionized (%)	Lag time (min)	Apparent permeability constant, ($min^{-1} \times 10^4$)
Salicylic acid			
1.25	1.75	18	3.00
2.74	35.46	18	1.90
8.23	99.99	No permeation	
Aminopyrine			
1.25	99.99	No permeation	
4.57	72.90	80	0.075
6.37	4.09	80	0.189

8) Catalogue by Japan Millipore Ltd., Tokyo, 1975.

Temperature Dependence of Drug Permeation

The temperature dependence of drug permeation was determined where the initial concentration of salicylic acid in the donor solution was of 1000 μg/ml at pH 1.76, as shown in Table III.

Table III. Temperature Dependence of Salicylic Acid Permeation

Temp. (°C)	Lag time t_0, (min)	Apparent permeability constant, P^*, ($min^{-1} \times 10^4$)	Diffusion constant D, ($cm^2/min \times 10^5$)
15	45	1.46	1.49
20	28	1.75	1.79
25	27	2.17	2.26
30	25	2.65	2.72
35	18	3.00	3.07

In this condition, most of salicylic acid was not ionized. Both P^* and D increased with the rise of temperature. It is known that temperature dependence of diffusion constant is expressed with Stokes–Einstein equation as follows:

$$D = R \cdot T / 6\pi\eta r \qquad (4)$$

where, D is diffusion constant, R is Boltzman constant, η is the viscosity of diffusion medium, r is the radius of the spherical particle and T is the absolute temperature. When $D \cdot \eta$ was plotted against T according to equation (4), a straight line relationship was obtained, as shown in Fig. 3.

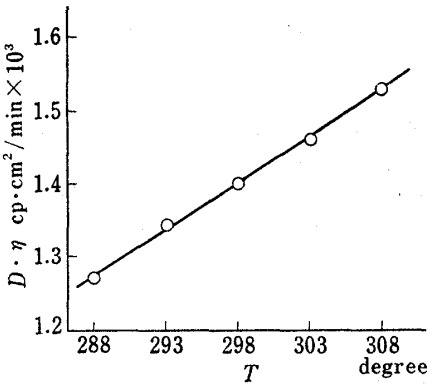

Fig. 3. Stokes–Einstein Equation Relationship D and T

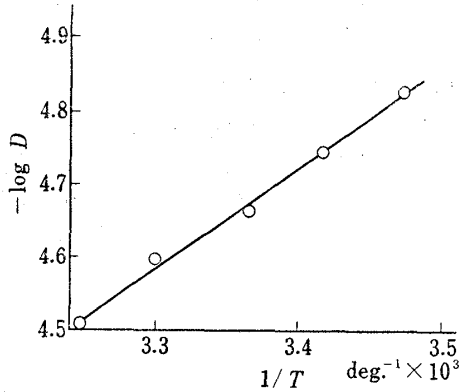

Fig. 4. Relationship between −log D and $1/T$

Table IV. The Effect of the Kind of Oils on the Permeation of Salicylic Acid

Oil	Lag time (min)	Apparent permeability Constant, ($min^{-1} \times 10^4$)	Distribution Constant, K	Viscosity (poise)
Olive oil	18	3.00	4.435	0.30
Liquid paraffin	210	0.21	0.325	0.32
Cyclohexane	7	5.81	0.205	0.0075
Oleic acid	3	6.39	5.670	0.18

The activation energy of diffusion of salicylic acid molecule was calculated from the slope of the plot of $-\log D$ against $1/T$ in Fig. 4, being found to be 6.27 kcal/mol. This result suggests that the permeation is a diffusion controlled one.

The Effect of the Kind of Oils on the Permeation of Salicylic Acid

In order to investigate the effect of the kind of oils in the Millipore membrane on P^*, the experiment was carried out in the initial concentration of salicylic acid 1000 μg/ml at pH 1.76 at 35°. P^* and also K clearly differed with the kind of oils, as shown in Table IV.

P^* increased with the increase of K and also might decrease with the increase of η.

As a result, a straight line relationship was obtained between P^* and K/η, as shown in Fig. 5. Actually, there was no remarkable difference in the viscosity of the oils used except cyclohexane. This result indicate that no special interaction between salicylic acid and oils.

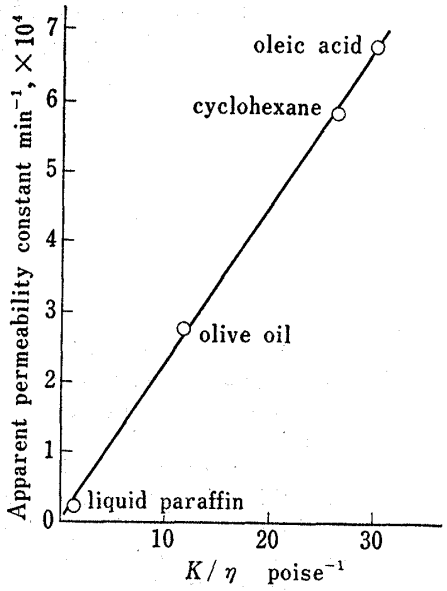

Fig. 5. Straight Line Relationship between P^* and K/η with Various Oils

Accordingly, the results mentioned above also suggests reasonably that the permeation of salicylic acid through the present membrane is controlled by a simple diffusion. The method employed in this study might give a useful means for obtaining an intrinsic diffusion constant as a basic parameter having relation to the drug permeation through a lipid layer, as might be more significant by finding a suitable oil material to saturate the membrane.