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Effect of ethanol on the true diffusion coefficient of diclofenac and its sodium salt in silicone membrane

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Abstract

The effect of ethanol on the diffusion coefficient of free diclofenac (DH) as a hydrophobic drug in a silicone membrane was studied and the value was compared with that of sodium diclofenac (DNa) as a hydrophilic material. The silicone membrane permeation of DH was described as a lipid pathway up to 60% w/w ethanol. The change in the partition coefficient of DH between the silicone membrane and ethanol-aqueous solution as a function of ethanol concentration seems to correspond with that of the partition coefficient of many drugs between octanol and water or a buffer. The apparent diffusion coefficient of DH and DNa in ethanol-aqueous solution was dependent on the ethanol concentration, i.e., it was related to the partition coefficient. The true diffusion coefficient of a lipid layer can be calculated by subtracting the partition coefficient from the apparent diffusion coefficient by changing the ethanol concentration.

Keywords: Diclofenac; Skin permeation; Lipid pathway; Diffusion coefficient; Ethanol effect; Partition coefficient

1. Introduction

One of the tactics for designing transdermal drug delivery systems involves a cosolvent of an ethanol-aqueous solution to increase the permeation of the drugs. High fluxes of nitroglycerin (Berner et al., 1989a,b), progesterone (Tojo et al., 1986), estradiol (Good et al., 1986), salicylic acid (Kurihara et al., 1990) and sodium diclofenac (DNa) (Nishihata et al., 1988; Obata et al., 1991) have been reported in transdermal systems using a cosolvent of an ethanol-aqueous buffer.

The influence of ethanol in aqueous solutions upon the transport behavior of several permeants has recently been investigated (Knutson et al., 1990; Kurihara et al., 1990; Ghanem et al., 1992). Based on the data for the permeability and partition coefficients, the diffusion coefficients in skin were determined, and were found to increase substantially with increasing ethanol concentration in the solvent. These results were interpreted to mean that ethanol altered the skin properties. The question then arises as to whether this phenomenon occurs through the silicone membrane in ethanol-aqueous solution as it does in the skin.

To examine the effect of ethanol on the diffusion coefficient in more detail, we selected free

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diclofenac (acid, DH) as a hydrophobic drug and DNa as a hydrophilic one, and studied permeation through a silicone membrane. The use of a silicone membrane facilitates estimation of the change in the membrane structure caused by the alcohol.

Polydimethylsiloxane sheeting (Silastic®) has often been used to investigate the diffusion of drugs (Twist and Zatz, 1986, 1988, 1990). It acts as a non-aqueous pore, a solution-diffusion membrane and, because of its hydrophobic nature, has a very much higher permeability for nonionized species. Because skin appears to act as a lipophilic membrane, it can be assumed that some basic physicochemical concepts can be examined using a lipophilic membrane such as Silastic®. It is important to be able to separate the change in a genuine membrane from the thermodynamic activity change in the donor phase, since ethanol may affect the skin and the solubility of drugs.

DNa is water soluble but readily penetrates through the abdominal skin in rats in vitro (Obata et al., 1993a) and in vivo (Nishihata et al., 1988; Obata et al., 1992) using an ethanol-aqueous solution as a vehicle. Additionally, we have reported on the experimental partition coefficient in octanol-water (Maitani et al., 1991), the calculated partition coefficient (Maitani et al., 1993b), the ionic behavior in an ethanol-aqueous solution (Maitani et al., 1993a), and permeation through a silicone membrane of diclofenac salts in aqueous solution (Maitani et al., 1994).

The purpose of the present report was to clarify the change in the diffusion coefficients of DH and DNa in ethanol-aqueous solution using a silicone membrane compared with that of DH using rat skin.

2. Experimental

2.1. Materials

DH was obtained by recrystallization twice from an ethanol-aqueous solution in an acidic state by adding hydrochloric acid to DNa (Sigma Chemical Co., St. Louis, MO, U.S.A.). Distilled water was used throughout the experiments. Ethanol was of guaranteed reagent grade (Wako Pure Chemical Industries Ltd). All other chemicals were of analytical reagent grade. Non-reinforced polydimethylsiloxane sheeting (Dow Corning, Midland, MI), Silastic® 500-1 (0.0127 cm thick), was used for the diffusive barrier. Silastic® 500-5 (0.0508 cm thick), of the same quality as Silastic® 500-1, was used for the absorption of the solute and solvent onto the membrane. The membranes were presoaked in water to remove extractables.

2.2. Vehicle solubility (C_w)

The saturation concentration of each solute in the applied vehicle solution was determined by suspending an excess of the solute in the solvent and allowing the mixture to stand for 1 week at 25 ± 0.05°C. The suspensions were centrifuged, then filtered (Ekicrodisc, 0.2 μm, Gelman Sciences Japan, Ltd, Japan), and assayed spectrophotometrically (Ubest-30, Japan Spectroscopic Co. Ltd, Tokyo, Japan).

2.3. Membrane solubility (C_p)

Membrane-solvent partition coefficients for each ethanol-aqueous solution system were determined in quadruplicate. Each accurately weighed sample (0.25–0.28 g) of the silicone membrane (0.0508 cm thick) was placed into a saturated aqueous solution of the solute. After the membranes had been maintained at 25 ± 0.05°C for 7 days (preliminary studies had shown that this was sufficient time to attain equilibrium), they were removed from the solvent and carefully rinsed with water to remove adhering solute. Complete extraction of solute from the membrane was achieved by immersion of the membrane in 5 ml of an ethanol/water (2:3 w/w) mixture and sonication for 15 min at approx. 50°C. The UV analysis of the extract solution yielded the total amount of solute present in the membrane.

2.4. Calculation of the nonionized fraction

The pK_a values of diclofenac were determined by the titration method in ethanol-aqueous

solutions at 25°C (Maitani et al., 1991). The nonionized fraction of DNa (F_u) was calculated from each pK_a value of diclofenac (Table 1) and the pH of the drug suspension in ethanol-aqueous solution using the Henderson-Hasselbalch equation:

$$F_u = 1/[1 + \text{antilog}(pH - pK_a)]$$

2.5. Calculation of the partition coefficient

The partition coefficient (P_{app}) was defined as the ratio of the equilibrium concentration of solute within the membrane to that in the solvent (C_p/C_w).

2.6. Solvent uptake into membrane

Previously weighed membranes were equilibrated with ethanol-aqueous solutions of varying weight percents of ethanol at 25°C for 7 days. The membranes were removed from each solvent, blotted dry with paper, and reweighed on an electrobalance. The absorption weight of solvent per dry weight of the membrane was measured. Each solvent in which the membrane was soaked was measured via a spectrophotometric assay at 275 nm where remaining solvent showed maximum absorbance.

2.7. Porosity

Considering the change in the membrane weight after solvent uptake into membrane, the porous part may be created, that is defined as the porosity (ϵ). The ϵ is calculated as:

$$\epsilon = \frac{W_s/\rho_0}{W_s/\rho_0 + W_{dry}/\rho_m} \quad (1)$$

$$W_s = W - W_{dry} \quad (1')$$

where W_s is the absorbed weight of the solvent at equilibrium, W and W_{dry} denote the weight of the wet membrane at equilibrium and the initial weight of the membrane, respectively, and ρ_o and ρ_m are the density of the solvent and the silicone membrane, respectively. The ρ_o values were obtained from a reference (International Critical Tables, 1928), and the ρ_m values were measured (1.4159 ± 0.0348 g/cm^3).

2.8. Permeation studies

Horizontal diffusion cells, maintained at 25°C, were used to perform the permeation studies as described in a previous report (Maitani et al., 1994). The donor and receptor solvent was 6 ml of the same weight percent of ethanol-aqueous solution. A suspension of DH and DNa was used as the donor solution. The available area of the silicone membrane for permeation was 0.968 cm^2, and its thickness was 0.0127 cm. 20 μl of the receptor solution was sampled. The concentration of solute in the sample was analyzed via HPLC. These studies were generally performed in quadruplicate.

2.9. Calculation of the permeability coefficient

The flux (J) through the silicone membrane was evaluated as the steady-state slope of a plot of the cumulative amount of DH and DNa per unit surface area vs time. The apparent permeability coefficient (K_{app}) was calculated from $K_{app} = J/C_w$ where C_w is the drug concentration in the donor solution.

3. Results

3.1. Permeability coefficient through the silicone membrane

It has been postulated that there are two types of pathway for drugs to permeate through the skin; a lipid pathway and a pore pathway (Blank, 1965). The silicone membrane has no aqueous pores, therefore, it may be reasonable to assume that the ionized form of DNa, for example, as an ion-pair and the nonionized form of DH may penetrate through it by means of a lipid pathway. K_{app} depends on the permeability coefficients for the nonionized form (K_n) and the ionized form (K_i) of DNa and depends on the fraction of the drug in each state according to Eq. 2:

$$K_{app} = K_n \cdot Fu + K_i \cdot (1 - F_u) \quad (2)$$

Table 1
The pH and drug ionized fraction ($1-F_u$) of saturated diclofenac sodium in ethanol-aqueous solution

DNa	Ethanol (w/w%)	pH [a]	pK_a [b]	$1-F_u$ [c]
	0	7.70	4.16	1.000
	19.9	7.82	4.69	0.999
	39.9	8.06	5.22	0.999
	59.9	8.51	5.75	0.998
	99.5	9.48	6.81	0.998

[a] The pH of DH and DNa suspension.
[b] Determined by titration at 25°C (Maitani et al., 1991).
[c] F_u was calculated using the pK_a value.

A DH suspension in ethanol-aqueous solution is not totally ionized and the nonionized form of DH may be able to penetrate through the silicone membrane. The ionized form of DH cannot form an ion-pair in distilled water, where there is insufficient counterion. The term $K_i \cdot (1-F_u)$ in Eq. 2 can be neglected; for the DH suspension, K_{app} values may be equal to the K_n values.

Table 1 shows the pH of the DNa suspension in ethanol-aqueous solutions and the $(1-F_u)$ values. The DNa suspension in ethanol-aqueous solution is more than 99.8% totally ionized. Since the $(1-F_u)$ values of DNa are almost unity in ethanol-aqueous solution, the term $(K_n \cdot F_u)$ in Eq. 2 can be neglected. The K_{app} value of DNa indicates that for the ionized forms of DNa, $K_{app} = K_i$.

Here, K is defined as K_n of DH and K_i of DNa and corresponds to the K_{app} values for DH and DNa suspensions in ethanol-aqueous solution, respectively. The K value is expressed as:

$$K = (1-\epsilon) \cdot P_m \cdot D_m / h \qquad (3)$$

where D_m is the apparent diffusion coefficient of the nonionized of DH and ionized forms of DNa in the membrane and h represents the thickness of the membrane. P_{app} for DNa is equal to P_m when F_u is close to zero and $P_m = P_{app}/(1-F_u)$. P_m is the partition coefficient of DH and the ionized form of DNa between the membrane and solvent.

Table 2 lists the C_w, C_p and log P_m values of saturated solutions of DH and DNa.

The permeation of DH and DNa through the membrane is characterized as a function of the weight percent of ethanol as shown in Fig. 1. The flux of DH from the saturated solution increased with increasing ethanol concentration. The flux of DNa decreased up to 60% w/w ethanol and then increased significantly in 99.5% ethanol.

To examine the solvent effect on the membrane, the amount of solvent absorbed in the membrane and the relative absorbance of the remaining solvent at 275 nm were plotted as shown in Fig. 2. These values increased with the ethanol concentration in the solvent.

Table 2
Physicochemical properties of diclofenac and its sodium salt

	Ethanol (w/w%)	C_w [a] (g/l)	C_p [b] (mg/g silicone)	log P_m [c]
DH	0	0.00113	1.251 ± 0.106	3.045
	19.9	0.00258	1.382 ± 0.142	2.729
	39.9	0.688	0.542 ± 0.057	−0.104
	59.9	1.181	1.528 ± 0.292	0.112
	99.5	73.413	2.684 ± 0.011	−1.437
DNa	0	13.202	0.384 ± 0.084	−1.536
	19.9	42.946	0.140 ± 0.016	−2.486
	39.9	80.484	0.054 ± 0.002	−3.176
	59.9	139.018	0.096 ± 0.010	−3.163
	99.5	55.989	0.093 ± 0.006	−2.779

[a] Solubility in water.
[b] Solubility in silicone membrane ($n = 3$–4).
[c] $P_{app} = C_p / C_w$. For DH, $P_m = P_{app}$. For DNa, $P_m = P_{app}/(1-F_u) = P_{app}$ as F_u is near zero. The molecular weights of DH and DNa are 296.15 and 318.13, respectively.

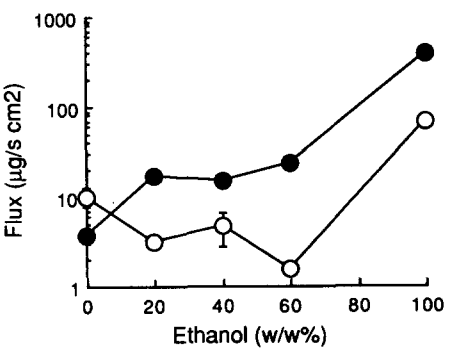

Fig. 1. Flux of diclofenac and its sodium salt through the silicone membrane at 25°C vs ethanol concentration (w/w%) from saturated solution. (●) DH, (○) DNa. Value without bar indicates that each S.D. is within the circle ($n = 3$–4).

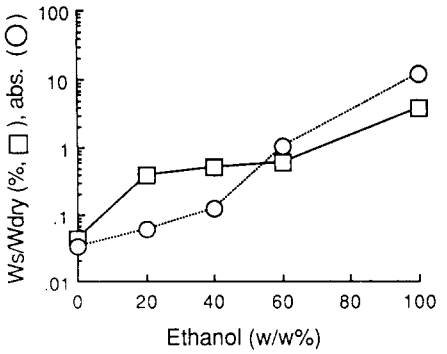

Fig. 2. Amount of various solvents absorbed for dry silicone membranes at 25°C from various ethanol-aqueous solutions and the relative absorbance of the solvent remaining at 275 nm. (□) Absorbed amount (W_s / W_{dry} in Eq. 1'), (○) absorbance of solvent. Value without bar indicates that each S.D. is within the circle ($n = 4$–5).

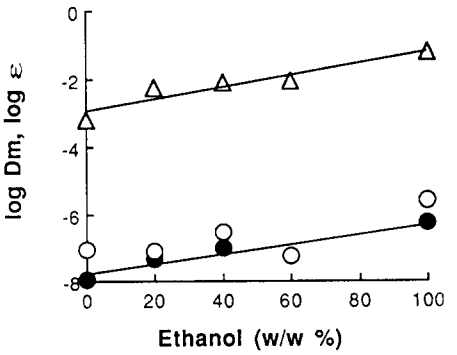

Fig. 3. Log D_m of diclofenac and its sodium salts and log ϵ of the silicone membrane vs wt% ethanol (f). The unbroken line was determined by the linear least-squares method using experimental mean values. (●) log D_m of DH, (○) log D_m of DNa, (△) log ϵ; for DH, log $D_m = 1.49 \times 10^{-2} \times f - 7.828$ ($r = 0.929$).

3.2. Increase in D_m values with ethanol concentration through the silicone membrane

The log ϵ and log D_m values of DH and DNa obtained using Eq. 1 are shown in Fig. 3. The log D_m values of DH and the log ϵ values of the membrane increased linearly with the ethanol concentration (wt%, f) ($r = 0.929$ and 0.945, respectively). However, the D_m values of DNa are almost equal except for that in 99.5% w/w ethanol.

The J, log K, log D_m and ϵ values are summarized in Table 3. The D_{mc}/D_{mw} ratio of DH through the silicone membrane (25°C) and rat skin (37°C) plotted against the ethanol concentration is shown in Fig. 4. The subscripts c and w indicate the ethanol-aqueous solution and water, respectively. The D_m values through the silicone membrane were calculated from the experimental P_m values used. The data for rat skin have already been reported by Obata et al. (1993a). In rat skin, the log P_m values between the skin and

Table 3
Flux, permeability and diffusion coefficients of diclofenac and its sodium salts and the porosity of the silicone membrane

	Ethanol (w/w%)	J [a] (μg/s cm^{-2})	log K [b] (cm/s)	log D_m [c] (cm^2/s)	D_m (cm/s)	R [d]	ϵ [e] ($\times 10^{-2}$)
DH	0	3.805 ± 0.454	−3.028	−7.970	1.072×10^{-8}	1	0.064 ± 0.058
	19.9	17.131 ± 0.775	−2.734	−7.357	4.395×10^{-8}	4.10	0.562 ± 0.054
	39.9	10.737 ± 0.970	−5.217	−7.007	9.840×10^{-8}	9.18	0.774 ± 0.029
	59.9	19.552 ± 3.618	−5.251	−7.256	5.546×10^{-8}	5	0.899 ± 0.164
	99.5	255.503 ± 35.86	−5.826	−6.256	5.546×10^{-7}	50	6.62 ± 0.390
DNa	0	9.424 ± 2.39	−6.670	−7.030			
	19.9	3.137 ± 0.129	−7.693	−7.102			
	39.9	4.793 ± 1.973	−7.781	−6.502			
	59.9	2.384 ± 0.707	−8.501	−7.234			
	99.5	68.243 ± 6.055	−6.470	−5.588			

[a] J ($n = 3$–4).
[b] $K = J \times 10^{-3} / (3600 \times C_w \cdot A)$; A, area of the membrane.
[c] $D_m = K \cdot h / \{P_m \cdot (1-\epsilon)\}$, $h = 0.0127$ cm.
[d] $R = \log(D_{mc}/D_{mw})$, where the subscript w refers to pure water as the donor solution without ethanol and the subscript c refers to the ethanol-aqueous solution.
[e] ϵ ($n = 4$–5).

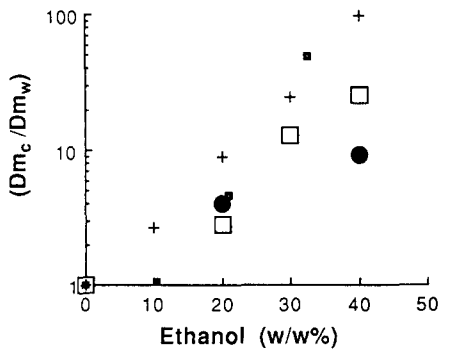

Fig. 4. The D_{mc}/D_{mw} ratio of diclofenac through the silicone membrane (25°C) and rat skin (37°C) plotted vs ethanol concentration. (●) DH through silicone membrane, (□) DH through rat skin (Obata et al., 1993a), (□) propranolol through cheek pouch (Egros et al., 1992), (+) estradiol through hairless mouse skin (Ghanem et al., 1992). The D_m values through the silicone membrane were calculated using experimental P_m values and those through rat skin using calculated P_m values from cohesion parameters (Maitani et al., 1993b). The D_{mc}/D_{mw} ratio indicates the apparent diffusion coefficient in ethanol-aqueous solution relative to that in water.

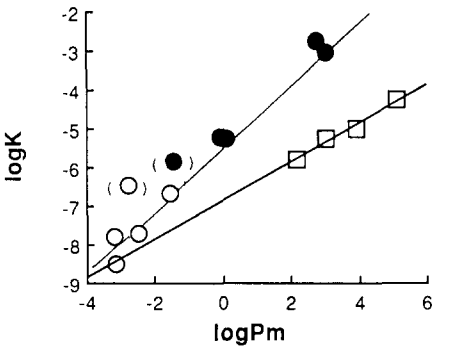

Fig. 5. Relationship between log K and log P_m of diclofenac and its sodium salt through a silicone membrane at 25°C and rat skin at 37°C. (●) DH across the silicone, (○) DNa across the silicone, (□) DH across rat skin (Obata at al., 1993a). The P_m values of silicone membrane were obtained experimentally from C_p/C_w values and those of rat skin were calculated from cohesion parameters (Maitani et al., 1993b). The unbroken line was determined by the linear least-squares method using experimental mean values except in 99.5% w/w ethanol. For DH across the silicone membrane: log $K = 0.803 \times \log P_m - 5.218$ ($r = 0.984$), across rat skin: log $K = 0.500 \times \log P_m - 6.868$ ($r = 0.990$).

solvent could not be measured because it is very difficult to measure the concentration of a drug in the skin. The P_m values were calculated using a cohesion parameter as reported previously (Maitani et al., 1993b). The D_{mc}/D_{mw} ratios of propranolol and estradiol are explained in the following section.

Fig. 5 shows plots of the log K and log P_m values of DH and DNa vs the ethanol concentration at 25°C. The log K and log P_m values of DH and DNa through the silicone membrane decreased with increasing ethanol concentration and show a linear relationship ($r = 0.984$) except in 99.5% w/w ethanol, and the slope of the line is not unity but 0.803. The log K and log P_m values of DH through rat abdominal skin in vitro (Obata et al., 1993a) are also shown in Fig. 5. The linear relationship between log K and log P_m of DH through rat skin is also shown ($r = 0.990$), but the slope of the line (0.500) is different from that in the silicone membrane.

To clarify the change in the log D_m values with ethanol concentration, the log K and log P_m values were plotted vs ethanol concentration as shown in Fig. 6. The values of both DH and DNa show a linear relationship with the ethanol concentration (for DH, $r = 0.866$ in log K and $r = 0.898$ in log P_m; for DNa, $r = 0.956$ in log K and $r = 0.930$ in log P_m), except in 99.5% w/w ethanol.

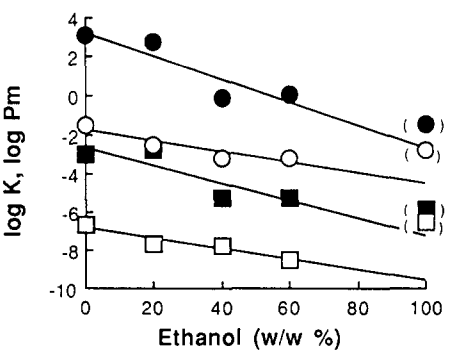

Fig. 6. Log K and log P_m vs wt% ethanol (f). (●) log P_m of DH, (■) log K of DH, (○) log P_m of DNa, (□) log K of DNa. The unbroken line was determined by the linear least-squares method using experimental mean values except 99.5% w/w ethanol. For DH, log $K = -4.576 \times 10^{-2} \times f - 2.685$ ($r = 0.866$); log $P_m = -5.816 \times 10^{-2} \times f + 3.190$ ($r = 0.898$). For DNa, log $K = -2.791 \times 10^{-2} \times f - 6.824$ ($r = 0.956$); log $P_m = -2.786 \times 10^{-2} \times f - 1.755$ ($r = 0.930$).

4. Discussion

4.1. Effect of ethanol on the silicone membrane

The weight of the soaked silicone membrane and the relative absorbance of the remaining solvent increased with the ethanol concentration (Fig. 2). Alcohols are reported to be capable of simultaneously forming hydrogen bonds with oxygen atoms in the polysiloxane backbone and undergoing nonpolar interactions, perhaps with the polymer side chains (Twist and Zatz, 1988). It might therefore be considered that ethanol interacts with the unreacted silicone oils and the cross-linking agent, etc., in the membrane, extracts them from the membrane, and forms the porous part. The solvent is then absorbed in the porous part. The ϵ values increased linearly with the ethanol concentration ($r = 0.945$). It is suggested that ethanol affects the silicone membrane and creates pores.

The effects of ethanol on permeation have been investigated by others (Liu et al., 1991ab). Ethanol may affect the skin as a result of many factors. Ethanol in a vehicle may function as an effective liquidizing agent for the lipid layer in the stratum corneum at low concentration (Knutson et al., 1990). At high concentration, ethanol may extract lipids from the skin and shrink keratin (Berner et al., 1989b). The porosity of the membrane may then be changed. The stratum corneum is a heterogeneous membrane, therefore it is difficult to distinguish these factors.

Furthermore, penetration through the stratum corneum is considered to involve two pathways, the lipid and polar pathways (Ghanem, et al., 1992). In the silicone membrane, only the lipid pathway may be predominant, and the change in structure of the membrane caused by ethanol is assumed to be simply estimated from the porosity. When ethanol affects the membrane and then creates pores, the polar pathway, which involves the transport through the pores, will occur in addition to the lipid pathway originally through the lipophilic part of the membrane.

Generally, however, the diffusion coefficient of the polar pathway in the silicone membrane may be much lower than that of the lipid pathway since the porosity is low in less than 60% w/w ethanol, and may therefore be neglected.

Based on the assumption that the ϵ values do not change markedly and the membrane is stretched in two dimensions, the thickness does not change greatly. Therefore, the h value is assumed to remain constant.

4.2. D_m value dependency on ethanol concentration through the silicone membrane and rat skin

The apparent diffusion coefficient of the lipid layer in the silicone membrane, D_m, should be constant on considering the ϵ value in Eq. 3. In addition, the D_{mc} values in an ethanol-aqueous solution should decrease with increasing ethanol concentration because the viscosity of the solvent increases with an increase in ethanol up to 40% w/w ethanol (Kagakubinran, 1982).

However, experimentally the D_m values of DH through the silicone membrane increased with ethanol concentration (Fig. 3). The D_{mc}/D_{mw} ratio of DH through the silicone membrane increased 9.18-fold in 40% w/w ethanol-aqueous buffer as shown in Table 3.

Ghanem et al. (1992) have reported that the change in the diffusion coefficient of a lipophilic drug, estradiol, through hairless mouse skin at 50% v/v (about 49.9% w/w) ethanol-aqueous solution increased almost 100-fold compared with aqueous solution as shown in Fig. 4. They used the ratio of D_{mc}/D_{mw} as an enhancement factor.

The D_m values of DH through the silicone membrane and the rat skin (Obata et al., 1993a) and those of propranolol through the cheek pouch (Egros et al., 1992) increase substantially with ethanol concentration compared with those in water as shown in Fig. 4.

One possibility of interpreting the phenomenon is that the D_m values are not calculated correctly. This may arise from the fact that the experimental P_m values do not correspond with the true diffusion coefficient (D). The P_m values of DH and DNa through the silicone membrane were obtained from C_p/C_w values experimentally, while those of DH through rat skin were calculated from cohesion parameters as reported

previously (Maitani et al., 1993b). These values may correspond with the real value according to some factors, and/or therefore the D_m values may still contain the factor of the partition coefficient. If so, the D values may possibly be calculated from the apparent diffusion coefficient D_m values obtained using Eq. 3.

4.3. Relation between log K and log P_m values through the silicone membrane and rat skin

The slope of the log K vs log P_m values of DH and DNa through the silicone membrane is 0.803 (Fig. 5). This means that the D_m values are not independent of the P_m values. We restate D_m in Eq. 3 using the D value. Therefore, the K values are expressed as:

$$K = (1-\epsilon)\cdot P_m^{\alpha}\cdot D/h = (1-\epsilon)\cdot P_m\cdot D_m/h \quad (4)$$

$$D_m = D\cdot P_m^{(\alpha-1)} \quad (5)$$

Here the α value is the slope of log K vs log P_m, 0.803 as shown in Fig. 5. The coefficient $(\alpha-1)$ accounts for the dependence of the D_m values on P_m values.

The P_m values in Fig. 5 are defined in two ways. One involves using many drugs from lipophilic to hydrophilic types. The other is defined by using a drug and changing the ethanol concentration in the ethanol-aqueous solution. Generally, the former is used.

Hatanaka et al. (1992) reported the partition coefficients (P) of 13 drugs in octanol/water and the permeability coefficients (K) across the silicone membrane and hairless rat skin. The relationships between log K and log P in the silicone membrane and hairless rat skin are shown, respectively, by the expressions:

$$\log K = 0.778\times\log P - 6.453$$
$$(\text{silicone}, r = 0.886) \quad (6)$$

$$\log K = 0.550\times\log P - 6.358$$
$$(\text{hairless rat skin}, r = 0.968) \quad (7)$$

On the other hand, using the partition coefficient of DH in the ethanol-aqueous solution across the silicone membrane and rat skin (Obata et al., 1993a), we obtained the following equations for log K and log P_m:

$$\log K = 0.803\times\log P_m - 5.218$$
$$(\text{silicone}, r = 0.984) \quad (8)$$

$$\log K = 0.500\times\log P_m - 6.868$$
$$(\text{rat skin}, r = 0.990) \quad (9)$$

Nevertheless, the definition of the partition coefficient is different; the slope of log K vs log P_m is almost the same in the silicone membrane (0.778 in Eq. 6 and 0.803 in Eq. 8), rat skin (0.550 in Eq. 7 and 0.500 in Eq. 9). This might demonstrate that the line of log K vs log P_m of one drug, DH, in the ethanol-aqueous solution is the same line of log K vs log P that many drugs in water or buffer fitted. Both the P defined by octanol/buffer and P_m values defined by ethanol-aqueous solution may be considered to show the same meaning.

4.4. Log K and log P_m dependency on ethanol concentration through the silicone membrane

The dependency of log K and log P_m values on the f value is examined. The values of log P_m and log K are expressed by:

$$\log P_m = a\cdot f + c \quad (10)$$

$$\log K = b\cdot f + d$$
$$= (b/a)\cdot\log P_m - (b\cdot c/a) + d \quad (11)$$

When the a value is high, this indicates that the P_m value is affected by ethanol. In the case of a low b value, this means that the K value is not affected by ethanol. In DH, the values of a, b, c and d are -5.816×10^{-2}, -4.576×10^{-2}, 3.190, and -2.685, respectively, as obtained from Fig. 6.

In Eq. 11, b/a and $(-b\cdot c/a + d)$ values are determined experimentally, since α is equal to b/a. From the slope and the intercept of log K vs log P_m in Fig. 5, the b/a and $(-b\cdot c/a + d)$ values are 0.787 and -5.218, respectively. The b/a and $(-b\cdot c/a + d)$ values are calculated to be 0.803 and -5.195 using the values from a to d. They are almost the same as the calculated values.

In DNa, the a and b values are almost identi-

cal, 2.78 and 2.79, respectively. This means that the log D_m value of DNa is independent of the ethanol concentration and may be equal to the log D value. The log D value of DNa is -6.967 which is calculated from the average log D_m values in Table 2 except for 99.5% w/w ethanol.

The log K and log P_m values of DH and DNa in 99.5% w/w ethanol deviate from those in 0–60% w/w ethanol (Fig. 5). This may suggest that partitioning of the lipophilic drug, DH, is not effective at the high ethanol concentration, since the P_m values of DH and DNa are almost equal (Fig. 6). As the porosity increases in the higher ethanol concentration, the pore pathway might become predominant, however, up to 60% w/w ethanol, the permeation of drugs is considered to be through a single lipid pathway.

4.5. True diffusion coefficient obtained from log D_m dependency on ethanol concentration through the silicone membrane

The D value may be calculated from Eq. 5 and 10 as:

$$\log D_m = \log D + (\alpha - 1) \cdot \log P_m$$
$$= (b - a) \cdot f + [\log D + (\alpha - 1) \cdot c] \quad (12)$$

Eq. 12 indicates that the D_m values increased with the increase of ethanol concentration in the solvent as shown in Fig. 3. The $b - a$ value is calculated to be 1.06×10^{-2} using the a and b values and is experimentally determined as 1.49×10^{-2} from the slope in Fig. 3. These values are almost identical. The log D values of DH are determined as -7.200 based on the intercept value of the line in Fig. 3, the α value in Fig. 2 (0.803) and c value (3.19). The log D values of DH and DNa are obtained as -7.200 and -6.967, respectively. The penetration pathway of DH and DNa does not seem to be different through the silicone membrane because DNa, a hydrophilic drug, penetrates through the silicone membrane as does DH, a lipophilic drug (Maitani et al., 1994). However, the mechanism of penetration of DH and DNa might be different, since the log D_m value of DNa did not depend on the ethanol concentration.

The slope of log D_{mc}/D_{mw} vs f (Fig. 4) may be expressed as $(b - a)$ in Eq. 12. The a and b values are negative ($\alpha = b/a$), therefore, the slope in Fig. 4, the $(b - a)$ value, will be high when the α value is low. Compared with the slopes in the silicone membrane and the rat skin, the latter is greater than the former as shown in Fig. 4. The α value of rat skin is lower (0.500 in Eq. 9) than that of the silicone membrane (0.803 in Eq. 8).

The high $(b - a)$ value may indicate that the diffusivity of the drugs in the pathway across the rat skin is more strongly affected by ethanol in the solvent than that in the silicone membrane. Perhaps this is due to the fact that ethanol can penetrate through rat skin easier than the silicone membrane due to the difference in polarity and structure of the membrane. In other words, this may indicate that drugs copenetrate through the rat skin with ethanol more readily than through the silicone membrane (Berner et al., 1989a; Liu et al., 1991b).

In the present study, the silicone membrane permeation of DH was described as occurring via a lipid pathway up to 60% w/w ethanol. At 99.5% w/w ethanol, ethanol decreased the partitioning of DH to the silicone membrane and also affected the membrane by increasing the porosity. Therefore, the permeation of DH might change from following a lipid pathway to a pore pathway. The permeability and partition coefficients of DH and DNa became almost identical at 99.5% w/w ethanol (Fig. 6).

The apparent diffusion coefficient of a permeant in ethanol-aqueous solution is concluded to depend on the ethanol concentration, but the true diffusion coefficient of the lipid layer can be calculated by subtracting the partition coefficient obtained from the apparent diffusion coefficient using a drug and changing the ethanol concentration.

This method of calculating the true diffusion coefficient will be useful in interpreting the permeability coefficient of drugs through the skin in ethanol-aqueous solution.

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