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Permeation of Sulfonylureas through Cellulose and Dimethylpolysiloxane Membranes[1,2]

HARUHISA UEDA,* NAOKI NAMBU, and TSUNEJI NAGAI

Hoshi Institute of Pharmaceutical Sciences, Ebara, 2-4-41, Shinagawa-ku, Tokyo, 142, Japan

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The permeation of sulfonylureas through cellulose and dimethylpolysiloxane membranes was investigated as part of a series of pharmaceutical studies on sulfonylureas.

In the case of cellulose membrane, the permeability decreased with increase in the molecular volume of sulfonylureas. The activation energy of permeation was close to that of diffusion of usual organic medicinals in water. The effect of pH on the permeation was negligibly small under the conditions used in this study. These results suggested that the permeation of sulfonylureas through cellulose membrane proceeds by diffusion through pores.

In the case of dimethylpolysiloxane membrane, at pH 2, the permeability constants, P, of tolbutamide, chlorpropamide, glyclopyramide and tolazamide were 16.15, 5.13, 2.33 and 0.95×10^{-8} cm^2·sec^{-1}, respectively, and the diffusion constants of the four drugs were all calculated to be $(14.92 \pm 1.39) \times 10^{-7}$ cm^2·sec^{-1}. The results for tolbutamide indicate that the rate of permeation of sulfonylureas through the membrane is pH-dependent because only the undissociated species can permeate through the membrane.

Keywords——sulfonylureas; cellulose membrane; dimethylpolysiloxane membrane; permeability constant; diffusion constant; lag time; dissociated state; undissociated state; diffusion through pore; partition-diffusion mechanism

Extensive studies on the absorption of tolbutamide from the gastro-intestinal (GI) tract have been reported.[3–7] It has been suggested but not confirmed that tolbutamide is absorbed by a passive transport mechanism, based on the pH-partition theory. Other sulfonylurea derivatives may resemble tolbutamide in absorption behavior from the structural point of view. However, no fundamental *in vitro* studies of sulfonylureas have appeared in relation to GI absorption or transportation of the drugs in biological systems. Generally, the membrane permeation of drugs has been extensively studied, and two kinds of model membranes. *i.e.*, cellulose and synthetic membranes, are often used. Cellulose membrane is useful in studying the interactions of drugs in permeation through the vital membrane by a simple pore-route diffusion mechanism.[8–10] On the other hand, synthetic membranes, such as dimethylpolysiloxane,[11,12] are useful in studying permeation primarily by the partition-diffusion mechanism.

Thus, the present study on the permeation of sulfonylureas was carried out as part of a series of pharmaceutical physicochemical studies of these drugs,[13] using two different membranes, *i.e.*, cellulose and dimethylpolysiloxane membranes. The other objective was to explore possibilities for controlling the rate of permeation of sulfonylureas through a non-polar membrane by physicochemical means. Such a study should give valuable insights into the possibility of clinical application of programmed release systems for sulfonylureas.

Experimental

Materials——Sulfonylureas used were the same as those described in the previous papers.[13a] Visking tubing No. 101 was obtained commercially to prepare the cellulose membrane. Dimethylpolysiloxane sheeting (Fuji Systems Co., Ltd., Tokyo) of av. 110—120 μm thickness was obtained commercially for the synthetic membrane. Silicone oil (Extra Refined Grade Dimethyl Silicone Fluid, 100 centistokes, Fuji Systems Co., Ltd., Tokyo) was used for the partition studies. The other materials used were of reagent grade.

Quantitative Determination of Sulfonylureas——In the neutral pH region, the sulfonylureas used were determined by the ultraviolet (UV) absorption method with a Hitachi 124 spectrophotometer at the wavelength reported previously.[13a] However, calibration curves for the low and high pH regions (pH 2.0—pH 5.0, and pH 10.0) were also prepared because the absorption wavelengths of sulfonylureas are affected slightly by change of pH.

Procedure and Apparatus for Permeation through a Cellulose Membrane——Permeation through a cellulose membrane was carried out according to the usual equilibrium dialysis method in a red-brown glass cell of the type described in the previous papers.[8,9] One compartment (A) contained 10^{-3} M of a drug in 1/30 M phosphate buffer solution (pH 7.0, unless otherwise stated), while the other (B) contained the same phosphate buffer solution only. The assembled cell was shaken mechanically at 30° (unless otherwise stated) in a Taiyo M-1 incubator. It was confirmed that the drugs were satisfactorily stable under the experimental conditions.

Procedure and Apparatus for Permeation through a Dimethylpolysiloxane Membrane——The permeation rate was measured in a cell similar to that reported by Nakano *et al.*[14] The cell was placed in a double beaker maintained at 30.0±0.5°. The diffusing solution in the bottom compartment of the cell was stirred at 600±10 rpm while the desorbing solution in the top compartment was also stirred at 300±10 rpm. A suspension of a drug was prepared by agitating an excess amount of the drug in KCl–HCl buffer (unless otherwise stated) with a magnetic stirrer at 30.0±0.5° overnight. Alkaline solution (pH 10.0) was used after prewarming as a desorbing solution to effect ionization of the permeant drug in order to maintain the sink condition. The alkaline solution used was a combination of 1/15 M disodium phosphate and 1 N NaOH. The usual alkaline buffers, such as borate and carbonate buffer solutions, were not used in this experiment, because such buffer solutions interfered with the quantitative determination of drugs by UV. The buffer capacity of the solution was not large, and the pH was affected by the permeant drug. However, a pH-meter was used to check that the pH of the solution was maintained over 9.0 during the experiment.

The diffusion cell was rinsed, then 57 ml of the suspension was poured into the bottom compartment and 40 ml of the alkaline solution was pipetted into the top compartment. The stirring of both compartments were started immediately after filling them with the solutions. At intervals, 2 ml aliquots of the desorbing solution in the top compartment were taken to determine the concentration. The same quantity of fresh alkaline solution was pipetted into the compartment.

Measurement of Solubility——Solubility at 30° was determined after filtration of the drug suspensions through a 0.45 μ membrane filter (TM-2, Toyo Roshi Co., LTD.).

Measurement of Partition Coefficient——In order to measure the partition coefficients of drugs between dimethylpolysiloxane oil and the aqueous buffer solution, five ml of KCl–HCl buffer solution (pH 2.0, ionic strength(μ)=0.1) containing *ca.* 5×10^{-5} M of the drug was mixed with 20 ml of dimethylpolysiloxane oil and shaken for 48 hr at 30°, and then the concentration in the aqueous layer was determined spectrophotometrically after centrifugal separation into two layers at 3000 rpm for 10 min.

Results and Discussion

Permeation through Cellulose Membrane

The permeation of a drug through a membrane has been expressed as follows[8]:

$$\log\{(C_0-2C)/C_0\} = -(2P\cdot S/2.303\cdot L\cdot V)\cdot t \quad (1)$$

$$P = f\cdot D \quad (2)$$

where C is the concentration in compartment B at time t, C_0 the initial concentration in compartment A, P the permeability constant, L the thickness of the membrane, f the membrane constant, D the diffusion constant, V the volume of solution in the compartments, and S the effective surface area of the membrane.

Theoretically, the permeability constant, P, does not vary with the initial concentration, C_0, as expressed by equation (1). Practically, however, P is known to be influenced by the concentration of solute molecules.[15] In this study, the values of P were obtained for the drugs with $C_0=10^{-3}$ M for convenience. The membrane constant, f, was taken as 0.0562 without experimental determination, because the cellulose membrane used in this study was identical to the one used by Nambu *et al.*[8] It was confirmed that a linear relation according to equation (1) was obtained for the data up to 18 hr using tolbutamide.

The values of permeability of each drug through cellulose membrane at 30° and pH 7.0 shown in Table I were obtained by measurement for 6 hr. The order of D was 10^{-6} (cm^2/sec), being close to those of sulfonamides[16] and phenothiazines.[8]

Table I. Permeability Constants and Diffusion Constants of Sulfonylureas through a Cellulose Membrane at 30°[a]

Compound	$C_0 \times 10^3$ (M)	$P \times 10^7$ (cm²/sec)	$D \times 10^6$ (cm²/sec)
Tolbutamide	1.01	2.09	3.71
Chlorpropamide	1.03	2.20	3.91
Carbutamide	1.00	1.98	3.52
Acetohexamide	1.02	1.86	3.32
Tolazamide	1.01	1.87	3.32
Glyclopyramide	1.04	1.92	3.42

a) $f=0.0562$, $V=200$ ml, $S=\pi \times 2.15^2$ (cm²), $L=0.0086$ cm in equations 1 and 2. Each value is the mean of three experimental runs.

The relationship between the diffusion constant, D, and molecular volume, Mv, is shown shown in Fig. 1. The concept of molecular volume was presented by Kopp,[17] and Arnold reported that Mv is closely related to the diffusion of the solute in the solvent.[18] In this study, the molecular volume, Mv was calculated according to the reference.[19] The deviation for carbutamide and glyclopyramide could not be explained clearly, but it was concluded that the diffusion constant decreased with increase in molecular volume.

The temperature dependence of permeation through a cellulose membrane is shown in Fig. 2. The values of activation energy, E_a, obtained from Fig. 2 are shown in Table II, and are close to those reported for diffusion-controlled dissolution.[20,21] In addition, it was demonstrated that the values of activation energy of sulfonylureas were a little smaller than those of phenothiazines.[8]

No marked effect of pH on the membrane permeation was observed at pH 7.0 and pH 7.8, as shown in Fig. 3. However, the effect of pH on the membrane permeation of tolazamide and acetohexamide at pH 6.2 seemed to be due to the low initial concentration, C_0, because their solubility was low in the test solution. In addition, about 50% of the molecules of tolazamide ($pK_a=6.18$) in the bulk solution existed in the undissociated state at pH 6.2,

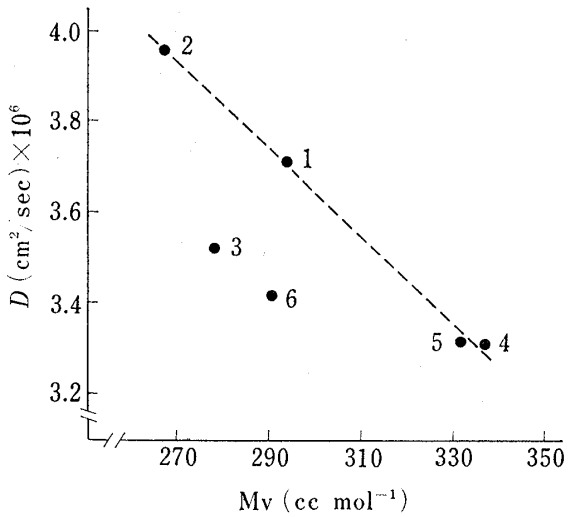

Fig. 1. Relationship between Diffusion Constant and Molecular Volume[17,18]

1: tolbutamide, 2: chlorpropamide, 3: carbutamide, 4: acetohexamide, 5: tolazamide, 6: glyclopyramide.

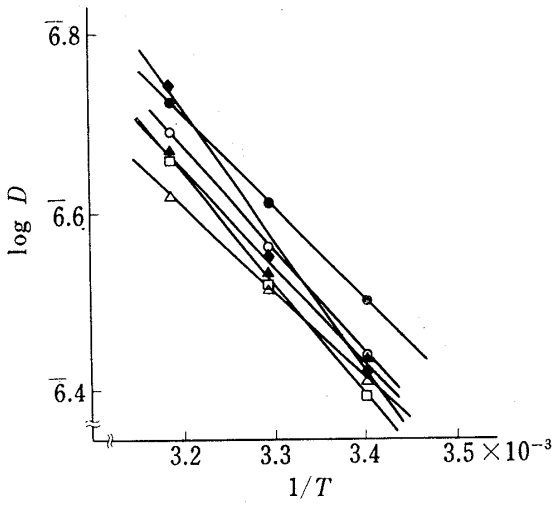

Fig. 2. Temperature Dependence of the Diffusion of Sulfonylureas through a Cellulose Membrane

○: tolbutamide, ●: chlorpropamide, △: acetohexamide, □: tolazamide, ▲: glyclopyramide, ◆: carbutamide.

Table II. Activation Energy of Permeation of Sulfonylureas through a Cellulose Membrane

Compound	E_a (kcal/mol)
Tolbutamide	5.30
Chlorpropamide	4.74
Carbutamide	6.82
Acetohexamide	4.56
Tolazamide	5.76
Glyclopyramide	4.93

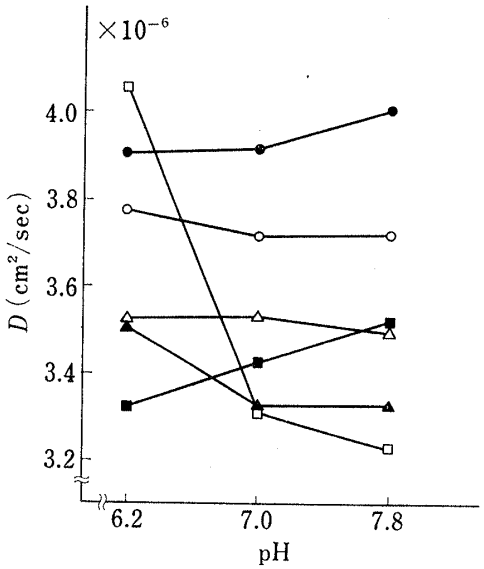

Fig. 3. pH Dependence of the Diffusion Constant of Sulfonylureas through a Cellulose Membrane at 30°

○:tolbutamide, ●: chlorpropamide, △: carbutamide, ▲: acetohexamide, □: tolazamide, ■: glyclopyramide

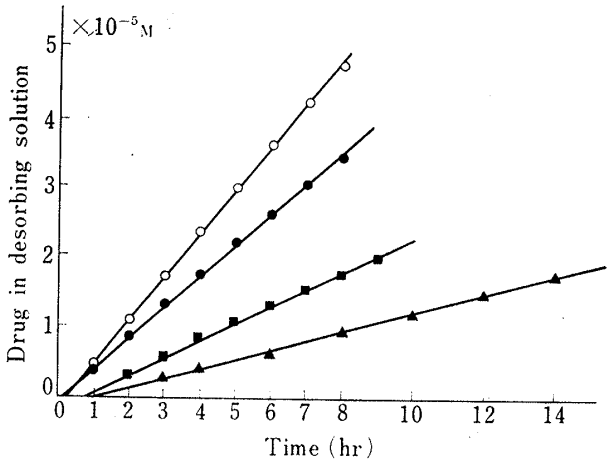

Fig. 4. Permeation Profiles of Four Sulfonyl ureas from Their Suspensions in KCl–HCl Buffer ($\mu=0.1$), pH 2.0, through a 120 μm Silicone Membrane(area = 13.85 cm²) into Alkaline Desorbing Solution (sink volume = 40 ml) at 30°

○: tolbutamide, ●: chlorpropamide, ■: glyclopyramide, ▲: tolazamide.

indicating that there was a little difference in diffusibility through pore route between molecules in the dissociated state and those in the undissociated state. In conclusion, the present results suggested that the permeation of sulfonylureas through cellulose membrane proceeds by diffusion through pores.

Permeation through a Dimethylpolysiloxane Membrane

Permeation profiles were obtained for only four sulfonylureas through the dimethylpolysiloxane membrane under the present conditions, as shown in Fig. 4. The reason why carbutamide and acetohexamide did not permeate may be as follows. In general, the rate of drug transportation through a dimethylpolysiloxane membrane is dependent on the form of the molecule, because only the undissociated form is soluble in the membrane and thus able to be transported. Carbutamide is an amphoteric electrolyte having an aromatic amino group, existing largely in the dissociated state in pH 2.0 solution. In the case of acetohexamide, the partition coefficient (0.039) between silicone oil and KCl–HCl buffer (pH 2.0) was nearly equal to that of chlorpropamide. On the other hand, the solubility (3.42×10^{-5} M) at pH 2.0 was less than one-tenth of those of the other drugs. Therefore, one of the reasons why acetohexamide did not permeate within the experimental period (24 hr) may be its low solubility. However, it is also possible that acetohexamide might be adsorbed strongly on the silica filler, as will be described later.

Table III. Solubility in KCl–HCl Buffer ($\mu=0.1$), pH 2.0, Partition Coefficient between Silicone Oil and KCl–HCl Buffer ($\mu=0.1$), pH 2.0, Permeability Constant through the Silicone Membrane, and Lag Time through 120 μm Silicone Membrane

Compound	Solubility 10^{-5} M	Partition coeff.	Permeability const. 10^{-8} cm^2 sec^{-1}	Lag time hr
Tolbutamide	37.51	0.098	16.2	0.16
Chlorpropamide	88.20	0.040	5.13	0.12
Glyclopyramide	100.5	0.016	2.33	0.48
Tolazamide	145.1	0.006	0.95	1.05

Each value is the mean of three experimental runs at 30°.

The permeability constant, P, was calculated from Fig. 4, according to the following equation for steady-state diffusion.[14]

$$\frac{dC}{dt_{ss}} = \frac{DS \cdot K}{l \cdot V} C_s = \frac{P \cdot S}{l \cdot V} C_s \qquad (3)$$

where $(dC/dt)_{ss}$ is the rate of increase in drug concentration in the desorbing solution in the steady state, D the diffusion constant in the membrane, S the effective surface area of the membrane, K the partition coefficient of a drug between the membrane and diffusing solution, C_s the drug solubility in the diffusing solution, l the thickness of the membrane, V the volume of the desorbing solution, and $P=D \cdot K$.

Permeability values (summarized in Table III) were all of the order of 10^{-8}(cm^2/sec), being close to those of amobarbital[12] and some benzodiazepines.[14] There was no apparent relation between lag time and the other parameters. The origin of the lag time in permeation through a dimethylpolysiloxane membrane has been discussed by Flynn *et al.*[22] in the cases of *p*-aminoacetophenone and ethyl *p*-aminobenzoate. They suggested that these phenomena were due to physical adsorption of the drugs by silica filler contained in the dimethylpolysiloxane membrane. The four sulfonylureas in this study do not have large differences in molecular size. Further, a specific interaction between each drug and the polymer matrix was not considered because a positive relationship between partition coefficient and permeability was seen (Table III). Therefore, the differences of lag time in this study may also be due to physical adsorption by silica filler in the dimethylpolysiloxane membrane. However, the mechanism will be investigated in more detail by studying the permeation of sulfonylureas through a fillerless membrane as well as the adsorption of sulfonylureas by colloidal silica from nonpolar solution.

It was pointed out by Nakano *et al.*[14] that liquid silicone oil may be employed in place of the polymerized silicone elastomer when relative values of partition coefficients are sufficient. A good correlation of permeability with partition coefficient between buffer solution and dimethyl polysilicone oil was obtained, as shown in Fig. 5. Since the permeability constant is a product of the diffusion constant and the partition coefficient, the diffusion constants of the four drugs were all calculated to be $(14.92 \pm 1.39) \times 10^{-7}$(cm^2/sec).

The effect of pH on the permeation is shown in Fig. 6. Since only a drug in the undissociated form can permeate into a dimethylpolysiloxane membrane, as described previously, the permeation rate of dissociative drugs may depend on the concentration of undissociated drug in the diffusing solution. The concentration of undissociated drug in solution, C_0, is usually given by:

$$C_0 = C_s \{1+\exp[2.303(\mathrm{pH}-pK_a)]\}^{-1} \qquad (4)$$

where pH is the pH of the drug solution, and K_a is the ionization constant of the drug. The pK_a of tolbutamide is 5.30 ± 0.04[23] and thus the molecules are almost all in the undissociated

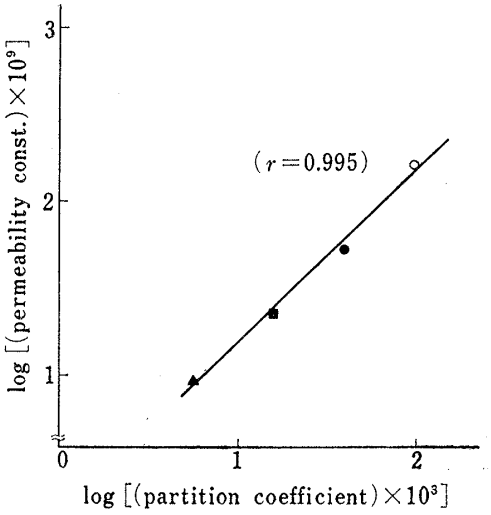

Fig. 5. Correlation of Permeability Constants through a Silicone Membrane at 30° with Partition Coefficient (silicone oil/KCl–HCl buffer (μ=0.1), pH 2.0)

○: tolbutamide, ●: chlorpropamide, ■: glycl-opyramide, ▲: tolazamide.

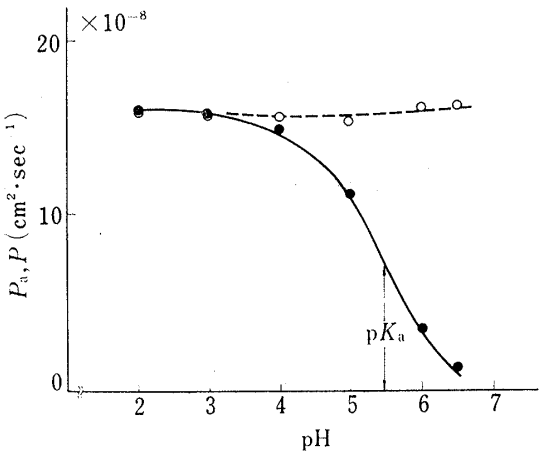

Fig. 6. Plot of Apparent Permeability Constant, P_a, and Permeability Constant, P, *versus* pH for Tolbutamide Solution

●: apparent permeability constant, p_a, calculated from eq. 3.

○: permeability constant, p, calculated from eq. 3 and eq. 4.

state at pH 2.0. However, the amount of dissociated tolbutamide molecules increases with increase of pH, thereby increasing the solubility. As shown Fig. 6, the apparent permeability constant, P_a, was calculated from Eq. 3 by using C_s, the total concentration of tolbutamide present in the diffusing solution. On the other hand, the permeability constant, P, calculated from the actual concentration of undissociated drug, was obtained from Eq. 4 and Eq. 3. The permeability constant, P, was $(16.01\pm0.48)\times10^{-8}$(cm²/sec) and did not vary significantly within the pH range examined. This constancy of P indicated that the only effect of pH on permeability was its effect on the concentration of undissociated drug.

These *in vitro* studies do not correspond directly to the phenomena in biological systems, but this kind of permeation study might give valuable insights into drug transport *in vivo* and the possibility of controlling the bioavailability.

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References and Notes

1) This paper forms Part XXXV of "Physico-chemical Approach to Biopharmaceutical Phenomena." The preceding paper, Part XXXIV: H. Ueda, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **28**, 3426 (1980).
2) A part of this work was presented at the 100th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1980.
3) A.A. Forist and T. Chulski, *Metabol. Clin. Exptl.*, **5**, 807 (1956).
4) W.L. Miller, Jr., J.J. Krake, M.J. Vander Brook, and L.M. Reineke, *Ann. N.Y. Acad. Sci.*, **71**, 118 (1957).
5) R. Konishi and S. Kimura, *Yakkyoku*, **24**, 975 (1973).
6) C.B. Tuttle, M. Mayersohn, and G.C. Walker, *Can. J. Pharm. Sci.*, **8**, 31 (1973).
7) G.D. Campbell, "Oral Hypoglycemic Agents," Academic Press, London and New York, 1969.
8) N. Nambu, T. Nagai, and H. Nogami, *Chem. Pharm. Bull.*, **19**, 808 (1971).
9) H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.*, **17**, 176 (1969).
10) M. Nakagaki, "Yakubutsu no Seitainai-Iko (Drug Transfer in Biological System)," Nankodo, Ltd., Tokyo, 1968.
11) M. Nakano and N.K. Patel, *J. Pharm. Sci.*, **59**, 77 (1970).
12) E.G. Lovering and D.B. Black, *J. Pharm. Sci.*, **62**, 602 (1973).

13) *a*) H. Ueda and T. Nagai, *Chem. Pharm. Bull.*, **26**, 1353 (1978); *b*) H. Ueda, K. Higashiyama, and T. Nagai, *ibid.*, **28**, 1016 (1980); *c*) H. Ueda and T. Nagai, *ibid.*, **28**, 1415 (1980).
14) M. Nakano, N. Kohri, Y. Arakawa, and T. Arita, *Chem. Pharm. Bull.*, **27**, 573 (1979).
15) National Research Council, U.S.A., "International Critical Table," Vol. V, McGraw-Hill Book Co., Inc., New York, 1929.
16) S. Yamabe, "Iyakuhin bunshiron," Asakurashoten, Ltd., Tokyo, 1968, p. 52.
17) H. Kopp, *Ann. Phys.*, **47**, 133 (1839).
18) J.H. Arnold, *J. Am. Chem. Soc.*, **52**, 3937 (1930).
19) K. Sato, "Busseiteisu suisanhou," Maruzen, Ltd., Tokyo, 1963, p. 150.
20) H. Nogami, T. Nagai, and A. Suzuki, *Chem. Pharm. Bull.*, **14**, 329 (1966).
21) H. Nogami, T. Nagai, and A. Kondo, *Chem. Pharm. Bull.*, **18**, 1185 (1970).
22) G.L. Flynn and T.J. Roseman, *J. Pharm. Sci.*, **60**, 1788 (1971).
23) M.J. Crooks and K.F. Brown, *J. Pharm. Pharmacol.*, **26**, 304 (1974).