Drug Release from the Water-in-Oil-in-Water Multiple Emulsion *in Vitro*. II. Effects of the Addition of Hydrophilic Surfactants to the Internal Aqueous Compartment on the Release Rate of Secretin

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For the application of water-in-oil-in-water (w/o/w) emulsions to a nasal dosage form of secretin, permeation tests were conducted *in vitro* to assess the effects of hydrophilic surfactants in the internal aqueous compartment on the release rate of secretin. The amount of secretin that permeated through an artificial membrane from a donor cell to a receptor cell was affected by the addition of sodium chloride or sodium alkylsulfonate to the internal aqueous compartment of w/o/w emulsions in the donor cell. Sodium chloride decreased apparent permeation rate constants from the internal aqueous compartment to the external aqueous phase (k'_1) as the difference in osmolarity between the internal compartment and the external phase increased. While sodium alkylsulfonates increased k'_1 in proportion to the values of (partition coefficient between the oil phase and the internal aqueous phase/partition coefficient between the oil phase and the external aqueous phase)/osmolarity ratio of the internal aqueous phase to the external aqueous phase. These results demonstrate that the release rate of secretin from w/o/w emulsions are affected by partition of the drug between aqueous and oil phases and by osmotic differences between the outer and internal phases.

Keywords secretin; w/o/w emulsion; hydrophilic surfactant; apparent permeation rate constant; partition coefficient; osmolarity

Introduction

In our previous studies,[1,2] we ascertained that it was feasible to develop the nasal dosage form of secretin. Secretin is a hormone secreted in the digestive tract and used clinically for the treatment of duodenal ulcers.[3,4]

There have been several investigations aimed at developing a nasal dosage form, a spray,[5] a gel,[6] or microspheres,[7] but few studies have been reported on the possible use of water-in-oil-in-water multiple emulsions (w/o/w emulsions) for nasal dosage.

On the other hand, in order to develop many potential applications, such as prolonged drug delivery systems,[8,9] there have been many studies on the release of the entrapped drug from w/o/w emulsions,[10,11] but few studies evaluating the release characteristics seen after the addition of hydrophilic surfactant to the internal aqueous compartment of w/o/w emulsions. Therefore, the evaluation of the release characteristics of secretin from w/o/w emulsions was considered to be important for the development of a nasal dosage form of secretin.

The objectives of this study was to assess the effects of the addition of hydrophilic surfactants to the internal aqueous compartment of the w/o/w emulsion on the release rate of secretin from the w/o/w emulsion droplets *in vitro*, in comparison with emulsions without additives used as control, and with emulsions containing sodium chloride. Furthermore, we attempted to elucidate how the physicochemical properties, such as partition coefficient and osmolarity, could contribute to the control of the release rate of secretin.

Experimental

Materials A 19000 CHR unit/mg (Crick, Happer and Raper unit) preparation of pork secretin (molecular weight = 3055, Eisai Co., Ltd., Tokyo), and Food dye Red No. 102, 2-hydroxyazonaphthalene-4',6,8-trisulfonic acid trisodium salt (new coccine, molecular weight = 604, San-ei Chemical Industries, Ltd., Osaka), were used as typical compounds in this study. Liquid paraffin (food additive grade in the Japanese Official Formulary) as an oil phase, sorbian monooleate (SMO 80, equivalent to Span 80®) as a lipophilic surfactant, and polyoxyethylene (20) sorbitan monolaurate (PSML 20, equivalent to Tween 20®) and polyoxyethylene (20) sorbitan monooleate (PSMO 80, equivalent to Tween 80®) as hydrophilic surfactants were from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). Sodium alkylsulfonates, such as sodium pentanesulfonate, sodium hexanesulfonate, sodium heptanesulfonate, and sodium octanesulfonate, as anionic hydrophilic surfactants, were from Tokyo Kasei Kogyo Co., Ltd., (Tokyo, Japan). These chemicals were used without further purification. Other chemicals employed were of analytical or reagent grade.

Dialysis membrane tubes were purchased from Spectrum Medical Industries, Inc. (U.S.A.), and polycarbonate membranes (0.4 μm in pore size, Nomura Micro Science K.K., Tokyo) were used as a test membrane.[12]

Preparation of Each Phase of w/o/w Emulsion For the preparation of the internal aqueous phases, each type of sodium alkylsulfonate, except for sodium octanesulfonate, was dissolved in the buffer whose composition was an isotonic 0.236 M citric acid: 0.123 M disodium phosphate aqueous solution (pH 6.33), and which was employed as the basic aqueous medium for both aqueous phases of w/o/w emulsions. The concentrations of sodium alkylsulfonates were adjusted to more than critical micelle concentration (cmc), whose values are shown in Table I. Sodium octanesulfonate was dissolved in buffer ranging in concentrations from 0.2 (less than cmc) to 1.0% (w/v) (more than cmc), and sodium chloride was from 0 to 1.8% (w/v). New coccine at 0.2% (w/v) or secretin at 0.064% (w/v) was dissolved in the internal aqueous compartment of the w/o/w emulsion.

SMO 80 was dissolved in liquid paraffin at a concentration of 10% (w/v) to prepare the oil phase whose basic formula was that described by Kawashima *et al.*[13] In our preliminary experiments, since the amount of secretin permeating from a donor cell to a receptor cell was too small to allow analysis of the concentration of secretin in the receptor cell when SMO 80 at 30% (w/v) was employed as a lipophilic surfactant, 10% (w/v) was employed as the concentration of SMO 80 in liquid paraffin. On the preparation of the external aqueous phase, PSML 20 was dissolved in the buffer to a concentration of 1.5% (w/v), since little coalescence was observed among the w/o/w emulsion droplets in the donor cell using microscope. For each of the w/o/w emulsions to be

TABLE I. Properties of w/o/w Emulsions to Be Tested

	Sample a	b	c	d	e	f	g	h	i	j	k
Additives	—	Sodium chloride			Sodium pentanesulfonate	Sodium hexanesulfonate	Sodium heptanesulfonate	Sodium octanesulfonate			
(cmc % w/v)					(1.001—5.007)	(0.104—1.035)	(0.501—2.005)	(0.302—1.008)			
% (w/v)	—	0.45	0.9	1.8	5	2	2	1	0.5	0.2	1
Diameter of w/o/w emulsion droplets (μm)	14.7	15.4	17.2	18.9	18.9	19.6	18.9	17.4	17.1	16.9	18.7
Specific surface area of w/o/w emulsion droplets (cm^2)[a]	4082	3896	3488	3175	3175	3061	3175	3448	3509	3550	3209
Diameter of internal aqueous compartment (nm)	164	178	184	210	208	172	185	187	212	185	180
Osmolarity[b] (internal aqueous ($mOsm/kg\ H_2O$) compartment)	297	425	561	835.5	886	507.5	475	389	339	314	392
(external aqueous phase)	299	299	299	299	299	299	299	299	299	299	299
$P_{o/i}/P_{o/e}$[c] (−)	1.26	1.19	1.92	1.56	5.03	2.89	10.96	14.08	3.74	1.22	14.08
Formation percentage (%)	95.02	98.77	98.31	97.61	97.15	98.48	97.82	97.77	97.41	99.17	97.04

a) Specific surface area is expressed in units per cm^3 of w/o/w emulsion droplet. *b*) The data are expressed as means obtained from triplicate samples. *c*) The ratio of the partition coefficients between the internal aqueous phase of w/o/w emulsion and the oil phase of the emulsion ($P_{o/i}$ to that between the external aqueous phase and the oil phase ($P_{o/e}$).

tested, the volume fraction of the internal aqueous compartment to w/o emulsion was 0.625, and that of w/o emulsion to w/o/w emulsion was 0.5.

The osmolarity of the aqueous phases of each type of w/o/w emulsion was determined according to a previously described method.[14]

Preparation The two-step emulsification was employed as a preparation procedure of w/o/w emulsions described in the previous study.[14]

In this study, 12.5 ml for the internal aqueous phase, 7.5 ml for the oil phase, and 20 ml for the external aqueous phase were adopted as the volume of each phase. The emulsification process for the first step, in which was prepared a single emulsion (w/o emulsion), and for the second step, in which the w/o/w emulsion was prepared, we employed a homogenizer (type X1020, Ystral GmbH, Germany) equipped with a U-shaped blade (7.3 mm diameter, 12.0 mm height) at the bottom of a cylinder (7.8 mm internal diameter, 10.2 mm external diameter) having several small lattice windows.

The mean diameters of the w/o/w emulsion droplets and the mean droplet size of their internal aqueous compartments were determined by the procedures as described in a previous study.[15] The specific surface areas of w/o/w emulsion droplets were calculated by using the mean diameters, and they are summarized in Table I.

Determination of Formation Percentage of w/o/w Emulsions When new coccine was used as the model compound, the formation percentage of the w/o/w emulsion was determined by the dialysis test described in the previous study.[14]

Secretin was observed to partition the aqueous phases into the oil phase in our preliminary study, and the formation percentage of the w/o/w emulsion was determined by the following procedure: about 10 ml of the fresh w/o/w emulsion were immediately centrifuged at 2800 rpm for 10 min. Then, 1 ml of the aqueous phase (the lower layer) was precisely withdrawn and diluted 10-fold with the external aqueous solution of w/o/w emulsion. The solution was then filtered with a Millipore filter (0.22 μm in pore size) and was analyzed by high performance liquid chromatography (HPLC).[12] When new coccine, which was not partitioned from the aqueous phase into the oil phase, was used in place of secretin, we confirmed that the data measured by this procedure described above were found to be almost the same as that measured by the dialysis test. In this study, experiments were conducted at 25 °C in triplicate. The mean values are shown in Table I.

Drug Permeation Test In order to monitor the release characteristics of model compounds from the w/o/w emulsion, drug permeation tests were conducted with the procedure described previously.[12]

Fifteen mililiters of the fresh w/o/w emulsion were poured into the donor cell, and 15 ml of the same solution used as the external aqueous phase of the emulsion were introduced into the receptor cell. The test was started by stirring the w/o/w emulsion in the donor cell at 300 rpm with a vertical semicircular shaped paddle, and the solution in the receptor cell with a magnetic stirrer at a constant rate. We have confirmed that 300 rpm was suitable for the permeation test because little coalescence was observed by microscope among the w/o/w emulsion droplets and the fluctuation range of the data measured in repeated permeation tests was found to be small (within 0.2%). For new coccine, about 1 ml of test solution was withdrawn periodically from the receptor cell and the concentration was determined by spectrophotometer.[14] The test solution was immediately replaced in the receptor cell. For secretin, aliquots of 50 μl of test solution were withdrawn periodically from the receptor cell and the concentration was measured by HPLC.[12] In this study, permeation tests were conducted at 25 °C in triplicate.

Permeation Tests of Secretin with o/w Emulsion The permeation test was conducted using the o/w emulsion prepared by the following procedure, to determine the apparent rate constant of permeation of secretin through the artificial membrane (polycarbonate membrane) between the donor and receptor cells.

Twenty mililiters of liquid paraffin, as in the oil phase, were added to 20 ml of the external aqueous solution. The mixture was then agitated at 2700 rpm with a homogenizer for 1 min in order to yield the o/w emulsion. Liquid paraffin was employed in the oil phase due to little partition of secretin from the external aqueous phase to the liquid paraffin. The volume fraction of the oil phase was taken as the sum of both the internal and the external aqueous phases of the w/o/w emulsion. Secretin was dissolved in the external aqueous phase of the o/w emulsion at a concentration of 0.064% (w/v).The permeation test was carried out according to the procedure described above.

The Apparent Partition Coefficients of Secretin between the Two Aqueous Phases of the w/o/w Emulsion and the Oil Phase of the Emulsion About 0.25 mg of secretin was precisely weighed into a glass centrifuge tube. The aqueous phase (5 ml) was poured into the tube and the secretin was dissolved. After the addition of 5 ml of the oil phase, the solution was shaken vigorously for 1 h and then centrifuged for 30 min at 2800 rpm. The tube was left to stand for 24 h, and a sample was withdrawn from the water layer. The sample was then filtered with a Millipore filter (0.22 μm in pore size) and was analyzed by HPLC.[12] The whole procedure was carried out at 25 °C in triplicate.

Results and Discussion

The release characteristics of secretin from the w/o/w emulsion were determined by the artificial membrane permeation test. In this experiment, secretin was employed as a model compound which was observed to partition into the oil phase from the two aqueous phases in these w/o/w emulsions.

The kinetic model for the permeation of secretin from the donor cell containing the w/o/w emulsion to the receptor cell is illustrated in Fig. 1. The internal aqueous

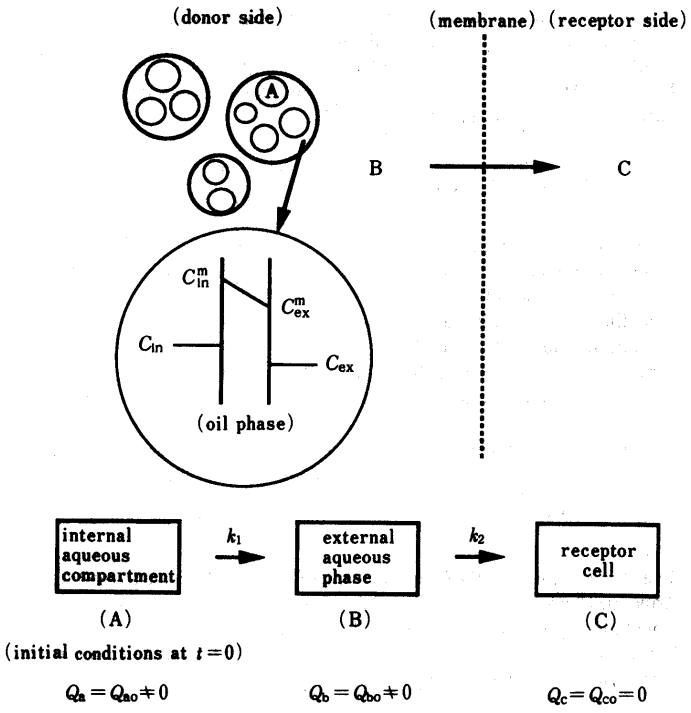

Fig. 1. Kinetic Model for the Permeation of Secretin from the Donor Cell Containing the w/o/w Emulsion to the Receptor Cell

Key: C_{in}, concentration of drug in the internal aqueous phase; C_{in}^{m}, concentration of drug in the oil phase partitioned from the internal aqueous phase; C_{ex}^{m}, concentration of drug in the oil phase partitioned from the external aqueous phase; C_{ex}, concentration of drug in the external aqueous phase.

compartment, the external aqueous phase and the receptor cell are defined as compartments A, B and C, respectively. Here, k_1 and k_2 represent the apparent rate constant of permeation from the internal aqueous compartment to the external aqueous phase, and that through an artificial membrane from the donor cell to the receptor cell, respectively. When a drug is assumed to permeate according to the pseudo first order kinetics, the following differential equations are proposed for the amount of drug in each compartment with respect to time (t min), based on the linear-compartment kinetic model,

$$dQ_a/dt = -k_1 Q_a \quad (1)$$

$$dQ_b/dt = k_1 Q_a - k_2 Q_b \quad (2)$$

$$dQ_c/dt = k_2 Q_b \quad (3)$$

where Q_a (mg), Q_b (mg) and Q_c (mg) denote the amounts of drug in compartments A, B and C, respectively. By integrating the above equations according to the initial conditions at $t=0$ ($Q_a = Q_{ao}$, $Q_b = Q_{bo}$ and $Q_c = Q_{co} = 0$), we obtain as below:

$$Q_c = Q_{ao}(1 + k_2/(k_1 - k_2)\exp(-k_1 t) - k_1/(k_1 - k_2)\exp(-k_2 t)) + Q_{bo}(1 - \exp(-k_2 t)) \quad (4)$$

Q_{ao} (mg) represents the initial amount of drug in compartment A at $t=0$, and Q_{bo} (mg) is the initial amount of drug in compartment B at $t=0$ which leaked out of the w/o/w emulsion during preparation. Equation 4 shows the amount of drug in the receptor cell (compartment C) which we have determined.

Then, the percent permeation of the drug, $\alpha(\%)$, in compartment C can be calculated according to the following equation,

$$\alpha(\%) = 100 Q_c/(Q_{ao} + Q_{bo}) \quad (5)$$

Furthermore, the k_2 value (2.14×10^{-4} for secretin), which was obtained from the permeation test using the o/w emulsion, was substituted in Eq. 4, and Eq. 6 was obtained as follows,

$$Q_c(\text{mg}) = Q_{ao}(1 + 0.000214/(k_1 - 0.000214)\exp(-k_1 t) - k_1/(k_1 - 0.000214)\exp(-0.000214t)) + Q_{bo}(1 - \exp(-0.000214t)) \quad (6)$$

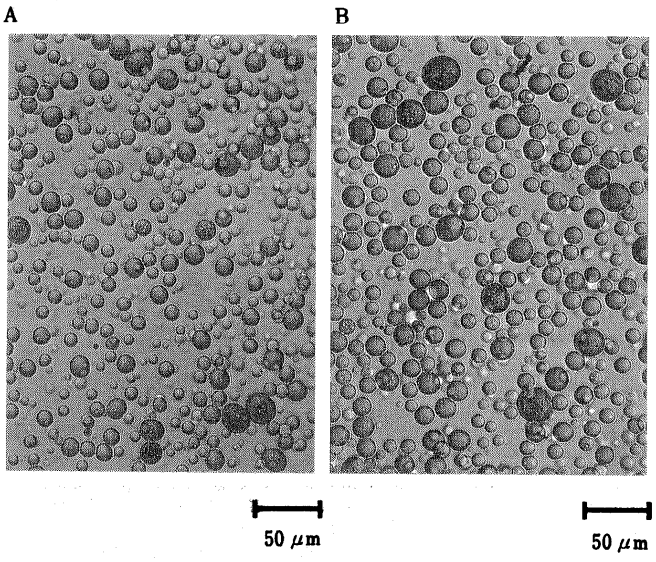

Fig. 2. Photomicrographs of the w/o/w Emulsion

A, before permeation test; B, after permeation test. The properties of the w/o/w emulsion in this figure are shown in Table I (h).

Figure 2 shows the photomicrographs of the w/o/w emulsion whose properties are shown in Table I (h). Photomicrograph (A) depicts the emulsion before the permeation test, and (B) is that after the test had proceeded for 7 h. The w/o/w emulsion droplets after permeation were observed to be slightly larger in diameter than those before the test. It would be attributed to the water uptake into the oil phase of the w/o/w emulsion but not coalescence on microscopic observation.[15]

The amount of secretin permeating from the donor cell into the receptor cell on the addition of sodium alkylsulfonates to the internal aqueous compartment of the w/o/w emulsion, as compared with that without sodium alkylsulfonates (control) is shown in Fig. 3. The amount of permeation for control was observed to be the smallest of all, and that with the addition of sodium octanesulfonate was the largest of all. These experiments demonstrated that the addition of sodium alkylsulfonates to the internal aqueous compartment increased the amount of secretin permeation, in comparison with the control, and that the amount of permeation could be controlled by the variety of sodium alkylsulfonate added.

Figure 4 shows the amount of secretin permeating from the donor cell to the receptor cell on the addition of sodium octanesulfonate at various concentrations to the internal aqueous compartment of the w/o/w emulsion, as compared with that without sodium octanesulfonate (control). The amount of permeation with the addition of

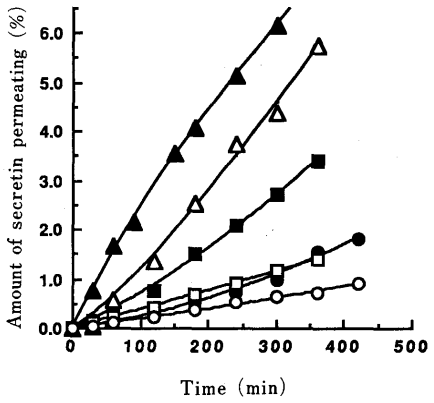

Fig. 3. Amounts of Secretin Permeating from the Donor Cell Containing the Various Kinds of w/o/w Emulsions to the Receptor Cell

Key: ○, control (without sodium alkylsulfonate); ●, sodium pentanesulfonate (5% w/v); □, sodium hexanesulfonate (2% w/v); ■, sodium heptanesulfonate (2% w/v); △, sodium octanesulfonate (1% w/v); ▲, data measured by using o/w emulsion. 10% (w/v) of SMO 80 was employed as a lipophilic surfactant in the oil phase of these w/o/w emulsions. The solid lines indicate the data calculated by the Eq. 6. The measured data are expressed as means ($n=3$).

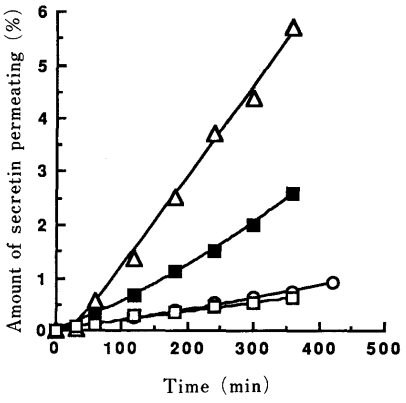

Fig. 4. Amounts of Secretin Permeating from the Donor Cell Containing the w/o/w Emulsions with the Addition of Sodium Octanesulfonate to the Receptor Cell

Key: ○, control (without sodium octanesulfonate); △, 1% (w/v); ■, 0.5% (w/v); □, 0.2% (w/v). 10% (w/v) of SMO 80 was employed as a lipophilic surfactant in the oil phase of these w/o/w emulsions. The solid lines indicate the data calculated by the Eq. 6. The measured data are expressed as means ($n=3$).

sodium octanesulfonate at 0.2% (w/v) was observed to be almost the same as the control, and was the smallest of all. The amount of permeation with the addition of sodium octanesulfonate at 1% (w/v) was the largest of all. It was thus found that as the concentration of added sodium octanesulfonate in the internal aqueous compartment increased, the amount of secretin permeating from the donor cell to the receptor cell rose.

Table II shows the effects of the addition of sodium alkylsulfonates to the internal aqueous phase of w/o/w emulsions on the apparent permeation rate constants (k_1) and those divided by the specific surface area of w/o/w emulsion droplets (k'_1), of secretin from the internal aqueous compartment to the external aqueous phase. The k_1 value was obtained from Eq. 6 by using non-linear least squares curve fitting (MULTI[16,17]). It is well-known that the k_1 is influenced by a specific surface area of w/o/w emulsion droplets. Hence, the k_1 value was divided by the specific surface area (shown in Table I), to obtain the k'_1 so as to assess the permeation rate of secretin independent

TABLE II. Effect of the Addition of Sodium Alkylsulfonate to the Internal Aqueous Phase of the w/o/w Emulsion[a] on the Apparent Permeation Rate Constants (k_1) and Those Divided by Specific Surface Area of w/o/w Emulsion Droplets (k'_1), of Secretin from the Internal Aqueous Compartment to the External Aqueous Phase

Sodium alkylsulfonate	$k_1 \times 10^{-4}$ (min^{-1})[b]	$k'_1 \times 10^{-7}$ ($cm^{-2} \cdot min^{-1}$)[c]
Control (without additives)	4.26	1.05
Sodium pentanesulfonate (5% w/v)	3.05	0.96
Sodium hexanesulfonate (2% w/v)	1.17	0.38
Sodium heptanesulfonate (2% w/v)	24.8	7.8
Sodium octanesulfonate (1% w/v)	68.9	19.98
Sodium octanesulfonate (0.5% w/v)	10.4	2.96
Sodium octanesulfonate (0.2% w/v)	0.17	0.04

a) 10% (w/v) of SMO 80 was employed as a lipophilic surfactant in the oil phase of these emulsions. *b*) The values of k_1 were calculated from Eq. 6 using the k_2 value ($2.12 \times 10^{-4} min^{-1}$) obtained from the permeation test of the o/w emulsion. *c*) Specific surface areas of w/o/w emulsion droplets are shown in Table I.

TABLE III. Effect of the Concentration of Sodium Chloride to the Internal Aqueous Phase of the w/o/w Emulsion[a] on the Apparent Permeation Rate Constants (k_1) and Those Divided by Specific Surface Area of w/o/w Emulsion Droplets (k'_1), of Secretin from the Internal Aqueous Compartment to the External Aqueous Phase

Concentration of NaCl (% w/v)	Osmolarity ratio[b] (−)	$k_1 \times 10^{-4}$ (min^{-1})[c]	$k'_1 \times 10^{-7}$ ($cm^{-2} \cdot min^{-1}$)[d]
0	0.993	4.26	1.05
0.45	1.421	0.88	0.23
0.9	1.876	−5.91	−1.69
1.8	2.794	−9.79	−3.08

a) 10% (w/v) of SMO 80 was employed as a lipophilic surfactant in the oil phase of these emulsions. *b*) Osmorarity ratio of the internal aqueous compartment of the w/o/w emulsion to the external aqueous phase of the emulsion. *c*) The values of k_1 were calculated from Eq. 6 using the k_2 value ($2.12 \times 10^{-4} min^{-1}$) obtained from the permeation test of the o/w emulsion. *d*) Specific surface areas of w/o/w emulsion droplets are shown in Table I.

of a surface area of w/o/w emulsion droplets.

The k'_1 values obtained after the addition of sodium octanesulfonate at 1% (w/v) were the largest of all, and those with the addition of sodium octanesulfonate at 0.2% (w/v) were the smallest. This indicates that the secretin entrapped with sodium octanesulfonate at 1% (w/v) was released most rapidly from the w/o/w emulsion droplets, and that at 0.2% (w/v) was hardly released. Also, k'_1, when sodium octanesulfonate was added, were decreased as the concentration of the additives fell.

In Figs. 3 and 4, the solid lines, calculated using Eq. 6 and the k_1 values in Table II, were fitted successfully to the measured data, which demonstrated the propriety of this theoretical equation.

It was demonstrated that the release rate of secretin from these w/o/w emulsion droplets was controlled by the addition of the various sodium alkylsulfonates and by varying concentrations of these substances in the internal aqueous compartment.

On the other hand, the release mechanisms, such as water flux caused by the difference in osmolarity between the internal and the external aqueous phases, carrier transport of inversed micelle,[18] and breakdown of the w/o/w emulsion droplets,[19] were reported in the w/o/w emulsion system. In order to clarify how the addition of sodium alkylsulfonates to the internal aqueous compart-

ment and the variation in their concentrations acted to control the release rates of secretin from the w/o/w emulsion droplets, the following studies were conducted.

Table III shows the effect of the concentrations of sodium chloride in the internal aqueous phase of w/o/w emulsions on the k_1 values and the k'_1 values of secretin from the internal aqueous compartment to the external aqueous phase. The values of k_1 and k'_1 were obtained by the method described above.

The values of k'_1 were decreased as the concentration of sodium chloride rose. It was supposed to be attributed to the water influx through the oil phase from the external aqueous phase to the internal aqueous compartment, caused by the increase with the difference in osmolarity between the two aqueous phases.[18] Also, the larger water influx could be considered to give the minus values of k'_1 at 0.9% (w/v) and 1.8% (w/v).

The release rate of secretin was found to be prolonged as the concentration of sodium chloride increased. However, when sodium alkylsulfonate was added, it was thought that the osmolarity was not the dominant factor controlling the release rate of secretin from the w/o/w emulsion droplets.

Table IV shows the effect of the addition of sodium octanesulfonate to the internal aqueous compartment of the w/o/w emulsion on the k'_1 values of new coccine or secretin from the w/o/w emulsion droplets. The value of k'_1, which was obtained from the permeation test entrapped new coccine alone in the internal aqueous compartment, was increased about 4-fold by the addition of sodium octanesulfonate at 1% (w/v) as compared with that without sodium octanesulfonate. In our preliminary study, k'_1 for new coccine was suppressed by the addition of sodium octanesulfonate at 1% (w/v) to the w/o/w emulsion whose oil phase contained 30% (w/v) of SMO 80. As a result of this experiment, it was supposed that the breakdown of the w/o/w emulsion droplets was increased as the stability of the emulsion fell owing to the reduction of the amount of SMO 80 added from 30% (w/v) to 10% (w/v),[20,21] because new coccine was found not to partition into the oil phase from the two aqueous phase.

On the other hand, k'_1 for secretin entrapped alone was increased by about 20 times by the addition of sodium octanesulfonate at 1% (w/v), over the value without sodium octanesulfonate, which indicated that the release rate was accelerated more than that of new coccine. Moreover, when secretin and new coccine were co-entrapped in the w/o/w emulsion droplets (Table I (*k*)), for each compound a k'_1 value was obtained similar to that found when they were entrapped separately. This indicated that the addition of secretin did not enhance the breakdown of w/o/w emulsion droplets. Also, the value of k'_1 for secretin should be almost the same as that for new coccine when secretin and new coccine are co-entrapped,[10] if the mechanism of release for secretin has been similar to that for new coccine. Whereas, k'_1 for secretin was found to be about 10-fold greater than that for new coccine. This result suggested that the mechanism of increase of k'_1 for secretin is different from that for new coccine.

Secretin was distributed from the aqueous phases to the oil phase in the w/o/w emulsion system (Table I). In addition, the partition coefficient of secretin was increased in proportion to the concentration of sodium octanesulfonate, but that at 0.2% (w/v) (less than cmc) was almost the same as in the control. This indicates that the micelle of sodium alkylsulfonates could participate in the partition of secretin from the internal aqueous compartment of the w/o/w emulsion to the oil phase of the emulsion. In Fig. 1, a schematic diagram of drug permeation through the oil phase dominated by the partition coefficients also appears. The amount of secretin permeation was considered to be dominated by the ratio of the partition coefficients between the internal aqueous phase of w/o/w emulsion and the oil phase of the emulsion ($P_{o/i}$) to that between the external aqueous phase and the oil phase ($P_{o/e}$) (Table I).

The relationship between k'_1 and $(P_{o/i}/P_{o/e})$/the osmolarity ratio of the internal aqueous phase of the w/o/w emulsion to the external aqueous phase of the emulsion (osmo) is shown in Fig. 5. The reason why the value of $P_{o/i}/P_{o/e}$ was divided by osmo was that the difference in osmolarity between the two aqueous phases was found to act as resistivity against the permeation of secretin from the internal aqueous phase to the external aqueous phase. Significant correlations, for k'_1 (correlation coefficient; 0.9767, $p<0.01$) was found to $(P_{o/i}/P_{o/e})$/osmo, which indicated that the $(P_{o/i}/P_{o/e})$/osmo was the dominant factor controlling the release of secretin from the w/o/w emulsion

TABLE IV. Effect of the Addition of Sodium Octanesulfonate to the Internal Aqueous Phase of the w/o/w Emulsion[a] on the Apparent Permeation Rate Constants of New Coccine or Secretin from the Internal Aqueous Phase of the w/o/w Emulsion to the External Aqueous Phase of the Emulsion (k'_1)

Compound	$k'_1 \times 10^{-8}$ ($cm^{-2} \cdot min^{-1}$)		
	Without sodium octanesulfonate	With sodium octanesulfonate (1% w/v)	
	(Separated entrapping)	(Separated entrapping)[b]	(Co-entrapping)[c]
New coccine	5.06	19.1	25.9
Secretin	10.5	200	247

a) 10% (w/v) of SMO 80 was employed as a lipophilic surfactant in the oil phase of these emulsions. *b*) The values of k'_1 were obtained from the individual permeation test for new coccine or secretin. *c*) The values of k'_1 were obtained from the permeation test entrapped new coccine and secretin together in the internal aqueous compartment of the w/o/w emulsion.

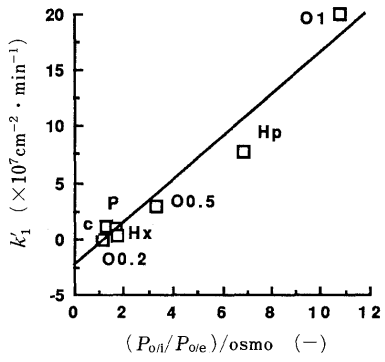

Fig. 5. Relationships between the Apparent Permeation Rate Constants (k'_1) of Secretin and the $(P_{o/i}/P_{o/e})$/osmo

Key: □, k'_1 ($y=-2.3600+1.8883x$, $r=0.9767$); c, control (without sodium alkylsulfonate); P, sodium pentanesulfonate (5% w/v); Hx, sodium hexanesulfonate (2% w/v); Hp, sodium heptanesulfonate (2% w/v); O_1, sodium octanesulfonate (1% w/v); $O_{0.5}$, sodium octanesulfonate (0.5% w/v); $O_{0.2}$, sodium octanesulfonate (0.2% w/v).

droplets.

The results of this study showed that the release of secretin from the w/o/w emulsion droplets with the addition of sodium chloride decreased as the difference in osmolarity between the two phases increased. However, as a result of the addition of sodium alkylsulfonates, the release of secretin was demonstrated not to be dominated only by osmolarity but also by the partition coefficients of secretin between the oil and aqueous phases.

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